UNDERSTANDING GRAPEVINE POWDERY MILDEW IN NEW YORK STATE: BIOLOGY, EPIDEMIOLOGY AND RISK ASSESSMENT

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Grapevine powdery mildew, caused by *Erysiphe necator*, is a cosmopolitan pathogen that threatens grape production worldwide. Accurate disease forecasts would enhance disease management, particularly where severity varies substantially between years, such as in New York State. Ascospore release occurred before local bud break of *Vitis vinifera* in NY, WA, NC and NJ, and was quantitatively related to accumulation of degree days and rain events during overwintering. Acute cold temperature events (<8°C) induced transient resistance in *V. vinifera* to *E. necator* that lasted for 24 to 36 h and reduced infection efficiency and colony development. Acute cold temperatures also damaged existing colonies, with the highest observed levels of mortality occurring in 3-day-old colonies. Occurrence of cold events as defined above was documented for many viticultural areas of the United States, Europe, and Australia. With respect to leaf surface temperatures, acute cold events probably occur more frequently than indicated in historical data bases of air temperature due to radiational cooling of the leaf surface during clear, calm nights, e.g., grape leaf surfaces in a Geneva, NY research vineyard were 0.9 to 6.6 degrees cooler than the air on clear nights. A regression model was developed from 23 years of historical weather and disease data to forecast fruit disease severity on unsprayed vines using the previous autumn's heat accumulation and pan evaporation (E_{pan}) rates (or E_{pan} estimated from evapotranspiration [E_{to}]) from 2 wks pre- to 6 wks post-bloom; timeframes relevant to production of (i) cleistothecia capable of surviving winter, and

(ii) conidial availability for infection of susceptible berries. Monte Carlo simulation coupled historical and forecasted weather data to predict a likely range of future E_{to} values, which were then used to predict favorability for disease. Powdery Mildew Risk Assessment models were developed using both logistic regression (LR) and recursive partition analysis (RPA) from historical disease and weather records, and classified risk for powdery mildew infection on fruit. The models correctly classified mild and severe years in 18 of 22 and 19 of 22 years in verification studies, and 9 of 10 and 5 of 10 years in validation studies for the LR and RPA models, respectively.

BIOGRAPHICAL SKETCH

Michelle Marie Moyer was born in 1982 in Normal, IL. In 1990, her family (mother, father and brother) moved to Stoughton, WI. Her father and younger brother own and operate Moyer's Landscape Services and Hometown Nurseries, INC, which was her first exposure to horticulture. She attended the University of Wisconsin-Madison, double majoring in Genetics and Plant Pathology. After a life-changing trip to Costa Rica in a Horticulture course run by Dr. James Neinhuis, she joined the forest pathology lab under Dr. Glen Stanosz for her senior capstone experience, and worked in the plant disease diagnostic lab with Dr. Brian Hudelson. She continued her plant pathology studies at Cornell University. Her stay at the New York State Agricultural Experiment Station and travels to the Riverland in South Australia only deepened her commitment to agriculture. Living in both of the great wine appellations (Finger Lakes and Riverland) also exerted a magnetic influence towards wine appreciation and culture, and she hopes to continue her involvement in the grape industry throughout her career.

"An education isn't how much you have committed to memory, or even how much you know. It's being able to differentiate between what you do know and what you don't."

–Anatole France (1844-1924)

"It is as important to address unquestioned answers as it is to address unanswered questions."

- As paraphrased from Dr. Nelson Shaulis (1914-2000)

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GENERAL INTRODUCTION

> "Wine is one of the most civilized things in the world and one of the most natural things of the world that has been brought to the greatest perfection, and it offers a greater range for enjoyment and appreciation than, possibly, any other purely sensory thing."
>
> - Ernest Hemingway, *Death in the Afternoon*

Grapes and wine are a source of passion for many people, throughout history and across the globe. The Romans transported vines on their conquests, and monks planted vineyards on the steep slopes of the Mosel. We head into the 21st century with a heightened sense of wine subculture with writers and bloggers influencing the often unreachable general population, rising economic status of the middle class, and the surge of medical research implicating health benefits of grape products (13, 21).

On a global scale, over 7.4 million hectares of grapes were harvested in 2008, with the USA contributing 379,360 of those hectares (20). The USA is the third largest exporter of grapes, the fifth largest exporter of grape juice and the seventh largest exporter of wine (20) in the world. Globally and in the USA, the vast majority of grapes grown are of the European grape species, *Vitis vinifera*, which contains the premium wine grape cultivars such as 'Chardonnay', 'Riesling', and 'Cabernet Sauvignon'. While New York State is the third largest producer of grapes in the USA (over 16,000 bearing hectares in 2007 (61)), *Vitis labrusca* type 'Concord' comprised 76% of New York's total production tonnage in 2008 (1).

A challenge in grape production is the control of diseases and pests. Grapevine powdery mildew, a disease caused by the obligate fungal biotroph *Erysiphe*

necator (syn. *Uncinula necator*, (Schw). Burr), is considered among the most important grape pathogens in the world (52). In wine-grape growing regions, powdery mildew is a double threat: in addition to reduced vigor and winter hardiness of infected vines (46, 49, 50), mild to severe fruit infection can cause a reduction in total soluble solids accumulation (60), and defects in juice and wine such as discoloration and faulty off-flavors (12, 28, 32, 42, 48). These defects can be detected from as little as 3% powdery mildew contamination on fruit (wt/wt) (53). Diffuse powdery mildew infection on berries, though not visible in vineyard assessments, is associated with increased levels of fruit rots, spoilage microorganisms, and an increased frequency of defects in wines prepared from such fruit (28, 32, 60).

ETIOLOGY AND EPIDEMIOLOGY OF GRAPEVINE POWDERY MILDEW

The disease cycle of *E. necator* (and powdery mildew epidemics) spans two growing seasons: disease levels and progress in year one influences epidemic development in year two.

Until the mid-1980s, cleistothecia of *E. necator* were thought to be non-functional, and mycelia within infected buds was widely assumed to be the sole source of primary inoculum in powdery mildew epidemics (56). These infected buds could give rise to ‘flag shoots’: shoots bearing a sparse to heavy aggregation of mildew colonies (49, 50, 55). These shoots only occur in regions characterized by relatively mild winter temperatures, such as California, Australia, the Mosel and Rhine River valleys of Germany, Italy, Spain and in southern France, to name a few (9, 11, 39, 44, 47, 50, 55, 56). In regions where winter temperatures are sufficiently low to kill the less winter-hardy infected buds (e.g. NY, PA, WA, and OH in the USA and irregularly in parts of Germany), flag shoots are not a source of primary inoculum (51). Cleistothecia are now known to be additional sources of primary inoculum in regions

where flag shoots are produced, and the sole source of primary inoculum elsewhere (11, 33, 34, 37, 40, 51).

Cleistothecia: Description, Development, and Role in Epidemics. The initiation of ascocarp development requires the presence of opposite mating-types in the field. Ascocarp initiation occurs at a hyphal fusion point between these two mating types (23, 25). In New York State, the first cleistothecia are typically found between late June and early July (23) on unsprayed vines, and are usually seen 1 to 2 months later in vineyards with a lower disease incidence. Plant resistance, as it delays development of foliar disease, also delays the time of cleistothecia initiation (23).

Ascocarp development is temperature dependent, with a maximum development potential between 16 and 25°C (25). Physiological maturation of cleistothecia, however, has slightly warmer temperature requirements. When temperatures are between 20 and 25°C, it takes 25 days for 50% of the cleistothecia population to reach physiological maturation, but at 16°C it takes 33 days to 50% maturity (51). While temperature appears to affect the rate of maturation, when temperatures are between 10 and 32°C, there is not an apparent effect on the total number of ascocarps produced (23).

Mature cleistothecia are almost spherical, with multiple appendages radiating outward from the ascocarp. Appendage tips are uncinate. Immature ascocarps are hyaline, changing from yellow to dark brown as they mature. Ascocarps generally contain six asci, measuring about 50-60 µm x 25-40 µm, and each ascus contains 8 ascospores when first formed. Two ascospores immediately degenerate and the most common number by the time of release is four ascospores per ascus. Ascospores are hyaline, ellipsoid and measure 15-25 µm x 10-14 µm. As cleistothecia age and reach the time where ascospore release may begin, the strength of the ascocarp wall begins to decrease but this weakening event occurs a few weeks prior to natural dehiscence

(24). The weakest point in the ascocarp wall is directly below the equatorial line, which is the breaking point during dehiscence (24).

At maturity, the hyphae/appendages that connect the cleistothecia to the parent colony die. This detachment and subsequent dehydration of the mature cleistothecium results in a change from a spherical shape to concavo-convex (23). Cleistothecia can subsequently be dispersed from the foliage of the grapevine to secondary substrates by rain. Many are deposited in bark crevices of cordons, heads and trunks of vines (10, 23). This dispersal period generally occurs between August and November in New York State (11). Ascocarps selectively transferred by rain to bark as they mature have exhibited a higher percentage of viability (45-75% in New York; 6-67% Washington) (34, 51), than those on fallen leaves, canes, or rachises (15-21% for leaves, 5% for fruit). Viable cleistothecia have not been recovered from vineyard soil. In drier climates such as California, South Australia and Eastern Washington State, cleistothecia surviving on leaf debris are also viable (34).

Temperature appears to play a role in the preparation of the ascocarp to dehisce. Those cleistothecia stored at 20°C showed a significant decrease in ascocarp wall strength as winter progressed into spring, whereas those stored at 4°C did not have a significant decrease in strength over the same time course (24). Similar observations have been made in the field (41). Cold temperatures (less than -20°C) can potentially reduce the viability of cleistothecia (15), but this study was done in a controlled environment with cleistothecia sampled early in their development.

While moisture does not appear to play a role in ascocarp development and maturation rate (33), it is a requirement for ascospore release (15, 24, 40, 41). When ascospores age, there is a decrease in water potential and a conversion of lipids to reducing sugars (24), resulting in increased osmotic pressure when ascocarps are wetted by rain events. Reported ascospore release occurs from budbreak through

bloom, (6-10 wks) and coincides with a period of rain or while leaves were wet after a rain event (11, 34, 41, 51). The minimum amount of rainfall associated with ascospore trapping under vineyard conditions in New York State was 2.5 mm coincident with temperatures between 6 and 24°C (24). In years where few rain events occurred between prebloom and fruit set, there was limited detection of powdery mildew in the field (29). Release occurred within 8 h of a rain event, with most ascospores detected within 6 h of a rain or wetting event. There have been reports of ascospore release lasting up to 72 h (40) in the laboratory.

Discernment of the source of primary infection in a vineyard, whether from ascospores or flag shoots is generally deduced from circumstantial evidence, as ascospore- and conidia-derived colonies are indistinguishable under field conditions. Such circumstantial evidence includes the distribution of colonies in the vineyard and their location within a canopy. Cleistothecia form within a generally continuous canopy and are uniformly dispersed to bark (10, 51, 59). Putative ascosporic colonies are typically located on the abaxial surface of basal leaves of shoots located near vine cordons and heads. These colonies are randomly distributed throughout the vineyard (34), rather than in intense disease foci as might be associated with flag shoots. However, if weather conducive to several secondary cycles of the disease occurs, such circumstantial evidence is insufficient to definitively identify a colony developing from ascosporic infection.

Asexual Development: Conidia Description and Colony Growth. The asexual spores of *E. necator*, conidia, play a key role in the propagation and spread of disease during the growing season. Conidial production is the driving force behind the often-considered explosive development in most powdery mildew lifecycles (57). In grapevine powdery mildew, conidia are derived from colonies initiated by mycelium

surviving in dormant buds of the grapevine (flag shoots) (50), from colonies initiated by ascosporic infection, and from colonies initiated by other conidia.

Historical literature cites flag shoots as the sole source of primary inoculum, but finding such shoots in the field is often challenging (23, 34, 37, 56). For example, in South Africa flag shoot formation is rare, and often a result of pruning methods applied to certain cultivars (37). Recent evidence strongly favors an early spring infection of young, susceptible buds, and which may be closer to previously infected and arising flag shoots (50, 54, 64). While the general location of the next year's flag shoots can be predicted, the actual infection of particular buds appears to be a random event (64).

Whether produced on flag shoots or by ascosporic colonies, conidia follow a general developmental pattern and response to environmental conditions. Morphologically, conidia are hyaline and 27-47 µm x 14-21 µm in size, and are borne in chains on multi-septate conidiophores. The terminal conidium is the oldest. Developing colonies have hyaline hyphae that measure about 4-5 µm in diameter. Appressoria are multilobed, and form a globose haustorium after penetration (49).

Conidial germination rate is at a maximum between 24 and 27°C (14) and takes around 30 h. Additionally, conidia that fail to form hyphae 48 h post germination will cease to develop (16). While germination of conidia can occur below 6°C, infection does not (14). After initial infection, colony growth rates increase with temperature, with a maximum growth rate and shortest latent period at 26°C (14). Conidia cannot infect leaves at temperatures above 32°C (14). Temperature can also play a role in conidia viability. At temperatures between 33 and 35°C, conidia have severely reduced germination rates: less than 10% in 5-10 h, and 1% in 10-20 h of incubation. At higher temperatures, there is some colony recovery after brief

exposures, but once temperatures reach 40.5°C for more than 6 h, colonies are killed (14).

The literature on the response of *E. necator* to moisture is varied. Early *in vitro* studies reported that moisture stress had relatively little impact on conidial germination (14). *In planta* studies, however, showed a strong correlation between humidity level (when between 10 and 84%) and conidial germination (5, 14). For growth on *Vitis* leaves, the optimum humidity for pathogen development and sporulation ranged from 83-86.5% relative humidity, a vapor pressure deficit of 450-520 Pa, and an absolute humidity between 18.5 and 23.4 g/m^3 (5). Free moisture, however, is detrimental to conidial germination (49, 57).

While the knowledge of basic powdery mildew biology is extensive, there are crucial gaps. Most of these gaps are in epidemic initiation and early-season disease development. While we have a good understanding of the conditions required for ascocarp dehiscence, we know relatively little about the season and ultimate duration of ascospore release. Research has not defined the period of ascospore release and the overwintering conditions that influence the timing of release. Second, there is little information on the effects of low temperature extremes on colony development. Logically, if acute high temperatures can damage existing colonies (7, 14), low temperatures might have a similar effect. Since the aforementioned high temperatures are a rarity in temperate regions like New York State, and low temperatures (≤6°C) are a common occurrence in the early portion of the growing season, it seems appropriate to develop a better understanding of how this low temperature range affects epidemic development.

MANAGEMENT STRATEGIES IN GRAPEVINE POWDERY MILDEW

Disease management strategies of commercial grape growers changed in the last 30 years, reflecting recent advancements and knowledge of powdery mildew biology (4, 22, 26, 27, 29, 30, 38). After establishing that cleistothecia were the primary source of overwintering inoculum in cool climates such as New York State and that grape tissue exhibits ontogenic resistance to powdery mildew, spray programs have shifted from late-season applications to an early-season focus (27, 29, 31). While these early season sprays can effectively control powdery mildew development on fruit, they often do not control season-long foliar epidemics (27, 29). Dormant, eradicant sprays with lime-sulfur or horticultural oils have demonstrated usefulness in reducing overwintering inoculum load and possible epidemic delay (31, 58), but their cost remains a limiting factor in grower implementation.

Chemical Control. Chemical control is still the most effective means for powdery mildew management on the commercial level. Sulfur, first discovered as a means for powdery mildew control in the 1850's (52), remains the most common fungicide in both conventional and organic management programs. Strobilurins (quinone outside inhibitors or QoIs: azoxystrobin, trifloxystrobin, pyraclostrobin, kresoxim-methyl), sterol inhibitors (demethylation inhibitors (DMIs): tebuconazole, penconazole, myclobutanil, fenarimol), carboxins (boscalid), quinolines (quinoxifen), benzimidazole (thiophanate-methyl), potassium salts, petroleum oils, and various combinations of such products are now frequently used in powdery mildew management and fungicide rotations. Unfortunately, many of the classes of effective fungicides have been prone to resistance development, or are at high risk for resistance development. Resistance to QoI fungicides has been documented in northeastern North America and appears to be qualitative in nature (63). Powdery mildew populations exposed to repeated applications of DMI fungicides have developed

quantitative resistance (17). Phytotoxicity issues can also arise when applying sulfur in combination or rotation with petroleum oils, and there are instances of sulfur phytotoxicity on native species and hybrid cultivars (2). Stylet Oil©, a specific petroleum oil, can also reduce total soluble solids in ripening grape berries if applied too close to harvest (35).

Biological Control. Biological control receives special consideration when discussing powdery mildew, as it is not a common practice used in vineyards due to the high value and low disease tolerance associated with the crop. Biocontrol agents rarely provide complete disease control, and often require frequent applications or specific combinations to optimize their effectiveness. Despite these limitations, biocontrol may play a larger role in powdery mildew management in the future as resistance to various fungicides develops or the demand for organic produce increases.

One such biological control agent is the mycoparasite *Ampelomyces quisqualis*, a common hyperparasite of many genera of powdery mildews. It infects existing mildew colonies, forms pycnidia, and after rainfall, releases conidia in the form of a cirrhus (18). While the natural population development of *A. quisqualis* many not be timed appropriately for powdery mildew control in a vineyard, studies have been done on culture and use of this mycoparasite as an introduced biocontrol agent. Cotton wicks cultured with *A. quisqualis* and suspended in the trellis at 15 cm shoot growth and again at bloom, reduced powdery mildew infections on foliage from 19.1% to 6.6%, and on the fruit from 38.2% to 15.7%, relative to controls (18). *A. quisqualis* can also parasitize cleistothecia, reducing the levels of primary inoculum going into the next growing season (19). However, parasitism must occur before cleistothecia are mature. The key to managing powdery mildew with this mycoparasite is early infection of the mildew colonies and a sufficient supply of free water (18, 19).

Another biocontrol agent is the mycophagus mite, *Orthotydeus lambi*. When released at high densities into vineyards at prebloom, *O. lambi* provided some suppression of powdery mildew on *V. vinifera* cv. 'Chardonnay' foliage and clusters (45). The exact mechanism of suppression is unknown, but grazing on the colony results in collapsed hyphal tissue (45). Unfortunately, mite populations can decline over time (45), suggesting that building populations to effective levels on the commercial scale may be difficult. These drawbacks, however, may not be as important when trying to control disease on North American grape varieties, which already demonstrate partial host resistance to powdery mildew (45). Suppression using *O. lambi* might have commercial application with such grape varieties.

Cultural Practices. Crop cultural practices, often in combination with fungicide treatments (8), also have potential in control of powdery mildew on fruit. One such method is basal leaf removal, which is a common practice vintners use to enhance the quality of the juice produced by the berries. Basal leaf removal can significantly reduce disease severity, but has no effect on disease incidence (8). Studies completed in New York, Washington, and South Australia have shown that leaf removal around the cluster zone can reduce powdery mildew by increasing sunlight exposure, but this can also have negative impacts on fruit quality, including severe sunburn (3).

BUILDING A DISEASE FORECASTING SYSTEM

The objective of modeling, as quoted by van der Plank (62), is to develop "a better understanding of the system." In plant pathology, disease models aim to mimic reality, incorporating real variables to quantitatively describe natural occurrences (62). Models exist for many biological processes, often incorporating very broad

developmental principles that can span multiple kingdoms, or specific nuances relevant to only one system.

The value of grapes, combined with the ubiquitous nature of powdery mildew, has made this particular disease a prime candidate for forecasting systems. Most existing models are concerned with describing the appropriate time intervals for spray programs or describing the rapidity of generation time (6, 36, 43, 56). Generally, these models are built on temperature requirements for powdery mildew development, almost entirely based on the seminal work Delp published in 1954 (14).

Unfortunately for disease modeling, the focus on management of one particular disease in a cropping system is rarely the reality. Vineyards can also suffer from black rot, downy mildew, botrytis, and sour rot. Control programs based entirely on the biology of powdery mildew may leave a gaping hole in vineyard management, making the system susceptible to assault by other pathogens. However, for research purposes, a singular focus is often needed, as the task of defining control systems to deal with multiple pathogens can be daunting (22). While this may seem contradictory to the concept of modeling, in order to complete the larger picture of disease management, a good understanding of all the inputs specific to a system are needed. As discussed by Gadoury (22), "…Eventually, specific models and recommendations must be reconciled to multiple pest systems if they are to have a positive impact on agriculture." The development of advisory and forecasting systems should be done in the context of rational decision making: science should provide tools for practical use, and it is up to the operator to decide which tools are appropriate for the job.

DISSERTATION RELEVANCE AND APPLICATION

The purpose of this dissertation was to develop a disease forecasting system for grapevine powdery mildew to be used by grape growers in New York State. By

also including investigations into the basic biology of *E. necator*, however, a more comprehensive foundation for understanding the epidemiology of powdery mildew was built, allowing for adaptation of such a model to regional or climatic scales. Chapters One and Two address knowledge gaps in the understanding of inoculum potential and roles of environment, specifically sub-optimal temperatures, in early-season epidemic development, respectively. Chapter Three presents a model for understanding potential epidemic outcomes and disease forecasting using large-scale environmental conditions as predictors. The model presented is by no means an absolute product, nor is it necessarily a "specific model" as referenced above (22). Its conception was an exercise in relating already described epidemic and weather relationships in an effort to pull out potential knowledge gaps in the grapevine-powdery mildew pathosystem. It provides a deterministic means of assessing weather conditions and the impact on powdery mildew development. The output of this model can be used to simplify the understanding of how environmental influences can alter epidemics, providing growers an additional tool to use in their overall vineyard disease management strategies. At the very least, its usefulness lies in its ability to portray the interaction between weather and disease outbreaks, and consequently provides a stronger basis upon which to make informed decisions. At the very most, it can be integrated with real-time and forecast weather data to provide predictions on the favorability for powdery mildew development at the desired scale of resolution. The simplicity of the model presented also allows it to be adjustable to suit regional climatic differences, and has the potential to be compatible with any form of delivery media preferably used to reach the target audience: students in a classroom, scientists in a lab, or growers in a field.

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CHAPTER ONE

SEASONAL RELEASE OF *ERYSIPHE NECATOR* ASCOSPORES AND EARLY DEVELOPMENT OF GRAPEVINE POWDERY MILDEW EPIDEMICS

ABSTRACT

In regions where winter temperatures are lethal to overwintering grapevine buds that have been internally colonized by *Erysiphe necator*, cleistothecia are the sole source of primary inoculum for powdery mildew. Knowledge of the distribution of ascospore release is based on a limited number of reports covering only a few geographic regions. In this study, ascospore release was monitored over multiple seasons in New York (NY), Washington (WA), New Jersey (NJ), North Carolina (NC), Virginia (VA), and Georgia (GA). In addition, release was monitored within these populations of cleistothecia overwintering in a common location, New York State. The time of ascospore release in these populations was positively correlated with accumulated late winter/early spring wetting events (liquid precipitation >2.5 mm and maximum temperatures >0°C) and heat unit accumulation (degree days base 0°C) (slope coefficients significant at $P<0.0001$). Overwintering cleistothecia subjected to laboratory assays that induced ascospore release reached 50% ascospore depletion before the date of local budbreak of *Vitis vinifera* in 85% of the site/yr combinations. Time and intensity of initial ascosporic infection were simulated by inoculating foliage at varying stages of canopy development between budbreak and bloom. Subsequent cluster infection was then assessed at verasion, across a spatial grid from the initial focus of inoculation. In 2008 when weather conditions were less conducive for powdery mildew, early foliar infection resulted in severe disease development on adjacent fruit. In 2009, when in-season weather conditions were favorable for powdery mildew development, the time of initial primary foliar infection did not affect the level of subsequent fruit infection, as both early and late infections resulted in near 100% incidence on fruit and high levels of disease severity. There was a steep disease gradient from focal points, with the highest levels of severity within 1.5 linear meters of the disease foci.

INTRODUCTION

Grapevine powdery mildew, caused by the fungal obligate biotroph *Erysiphe necator* (syn. *Uncinula necator* (Schwn.) Burr), is a global threat to vineyards virtually irrespective of climate or locale (29). *Erysiphe necator* can overwinter in vineyards in two ways: as mycelium in dormant infected buds and as cleistothecia borne on fallen leaves or deposited on the bark of the vine (3, 4, 22, 23, 25, 26, 30, 31, 34, 36, 37). Mycelia in dormant buds give rise to densely colonized shoots the following spring, more commonly referred to as flag shoots (30) . Despite intensive scouting efforts flag shoots have not been found in New York (31), presumable due to the reduced cold hardiness of infected buds (30-33), and cleistothecia are therefore the only confirmed source of primary inoculum in New York (31). Clarification of the role of ascosporic inoculum (1, 3, 11, 22, 23, 25, 31, 37) and quantification of ontogenic resistance in berries (8-10, 16, 19, 21), has led to a shift in the focus of control programs towards suppression of ascosporic infection and protection of fruit during the critical period of high berry susceptibility (6, 15, 20).

Ascocarp maturation, dehiscence and ascospore discharge in *E.* necator have been extensively studied (2, 4, 7, 11-14, 26, 27), and a general rule-of-thumb was proposed and widely deployed to describe the conditions under which cleistothecia would release ascospores and would also be suitable for infection: 2.5 mm or more of rain coincident with temperatures at or above 10ºC. With respect to ascospore release, many of these studies were limited to only a few years (e.g. three or less (3, 12, 22)) and a low number of sites (e.g. often one (12, 22)). Some were restricted to laboratory studies (12, 26) or focused on ascospore release during the growing season of grapevine from late dormancy through bloom (1, 3, 12, 23, 27). Trapping of ascospores in vineyards was often conducted under conditions where airborne ascospore concentration was near the threshold of detection for the methods reported

(12, 23). These studies add breadth to our knowledge of ascospore release under vineyard conditions, but they are largely confirmatory in that they show that ascospores can be detected under similar conditions among diverse regions. However, none is sufficiently comprehensive to precisely describe potential variation in the distribution of ascospore release.

The objectives of this study were to evaluate how overwintering climatic conditions and changes in these conditions influence ascospore release, to delineate the seasonal duration of ascospore release, and to determine how the timing and quantity of primary inoculum impacts powdery mildew infection on grape clusters.

MATERIALS AND METHODS

Cleistothecia collection, overwintering, and discharge assessments.

Effects of overwintering conditions on ascospore release. Cleistothecia from New York (NY), New Jersey (NJ), North Carolina (NC), Washington (WA), Georgia (GA) and Virginia (VA) were collected and overwintered in both their location of origin and in NY (Table 1.1). Reciprocally, New York populations were overwintered in all the foregoing locations unless otherwise noted. For all populations, field-grown grapevine leaves bearing dense aggregations of mature ascocarps (31) were collected in late September or early October (prior to leaf fall). Leaves were immediately rinsed with distilled water over stacked Cobb sieves (US Standard Sieves, No. 50 over No. 120, with 0.297 and 0.125 mm mesh openings, respectively) to collected cleistothecia (2). Cleistothecia were suspended in dH_2O, and placed on 9 cm filter disks using 10 ml aliquots, and filtered with a vacuum pump (Will Scientific, Inc., Rochester, NY, USA) at 10 kPa until a final concentration of 500 cleistothecia/disc was reached. Filter discs were air dried, folded in quarters, and stapled to white-painted pine boards (2.5x25x60 cm) as shown in Fig 1.1. In 2005, cleistothecia were collected from the

Vitis interspecific hybrid 'Chancellor' at the New York State Agricultural Experiment Station (NYSAES) in Geneva, NY. In 2006, cleistothecia were collected from NYSAES ('Chancellor'), NJ (*V. vinifera* 'Cabernet franc'), WA (*V. vinifera* 'Chardonnay'), NC ('Chardonnay'), VA (*V. vinifera* multiple cultivars), and GA (multiple *Vitis* interspecific hybrid cultivars). In 2007, cleistothecia were collected in NY, WA, NC, and VA as described for 2006. In 2008, cleistothecia were only collected from 'Chancellor' grapevines in NY. In 2005, 2006 and 2007, leaves from NC, WA and GA were shipped to NY, stored at 4°C for up to 3 days, and the cleistothecia were removed and overwintered as above. Cleistothecia from leaves collected in NJ and VA were transferred to filter paper at their respective sites as described above.

Figure 1.1- Filter discs bearing cleistothecia of *Erysiphe necator* were overwintered outdoors on pine boards pained white. Boards were placed in locations away from buildings, with the filter disc side facing south.

Boards bearing the filter paper discs were stored at 4°C for 1 to 4 days before they were placed at their designated overwintering vineyards, filter discs facing south, or shipped to their overwintering locations (NC, NJ, VA, GA and WA) overnight. Boards were (i) mounted directly to vineyard posts, or (ii) freestanding using back-mounted 1.25 cm galvanized steel pipes staked into the ground. A summary of collection dates, location of collections and time of placement of cleistothecia in the field is provided in Table 1.1.

Starting on the dates indicated in Table 1.1, three filter discs (subsamples) were collected for laboratory assays of ascospore discharge every two weeks until March, then weekly thereafter. Cleistothecia overwintered in locales other than NY were shipped overnight to NYSAES for the above assays (APHIS Permit No. P526P-07-04968). A 1-cm diameter disc was cut from each overwintering filter disc using a cork borer, and placed, cleistothecia exposed, on the lid of a Petri plate that was lined with two water-saturated 9-cm filter discs. A glass microscope slide was placed in the bottom half of the Petri plate, and the lid of the plate was replaced such that the cleistothecia were suspended over the glass slide (Fig. 1.2). The plates were incubated at room temperature (22-25ºC) for 24 h under 16 h light/8 h dark, and the glass slides were removed from the plates, stained with 0.05% Cotton Blue in lactoglycerol, and visually inspected at 100X under a compound microscope (Leica DMLB, Germany). The total number of cleistothecia on the filter discs and total number of ascsospores on glass slides (germinated and ungerminated) were recorded for each subsample. The percentage of the season's total ascospore release from each site (normalized for the total number of cleistothecia in each sample replicate sample 1-cm disc, averaged over the three replicate samples per collection date, and calculated as a running total) was compared to the number of ascospore release events, i.e., 2.5 mm of rain coincident

with daily maximum temperatures above 0°C (12), and accumulated degree days (base 0°C), starting 1 January for each year.

Table 1.1- *Erysiphe necator* cleistothecia collection dates, locations, and wintering sites for 2005-08.

Year[x]	Collection Location	Overwinter Location	Date Collected	Date in Field	First Sample Date	Last Sample Date
2005	Geneva, NY	Geneva, NY	24 Sept	29 Sept	12 Jan 06	30 May 06
		Prosser, WA	24 Sept	1 Nov	18 Jan 06	29 May 06
		Raleigh, NC	24 Sept	1 Nov	9 Jan 06	30 May 06
		Chatsworth, NJ	24 Sept	1 Nov	9 Jan 06	30 May 06
2006	Geneva, NY	Geneva, NY	9 Sept	15 Sept	17 Nov	6 June 07
		Prosser, WA	9 Sept	2 Nov	15 Nov	13 June 07
		Raleigh, NC	9 Sept	15 Sept	15 Nov	13 June 07
		Chatsworth, NJ	9 Sept	10 Oct	17 Nov	7 June 07
		Winchester, VA	9 Sept	9 Oct	15 Nov	7 June 07
		Cleveland, GA	9 Sept	2 Nov	14 Nov	13 June 07
	Prosser, WA	Geneva, NY	24 Oct	17 Oct	17 Nov	6 June 07
		Prosser, WA	24 Oct	2 Nov	15 Nov	13 June 07
	Raleigh, NC	Geneva, NY	8 Sept	13 Oct	15 Nov	6 June 07
		Raleigh, NC	8 Sept	15 Sept	17 Nov	13 June 07
	Chatsworth, NJ	Geneva, NY	10 Oct	13 Oct	17 Nov	6 June 07
		Chatsworth, NJ	10 Oct	10 Cot	17 Nov	7 June 07
	Western VA	Winchester, VA	9 Oct	9 Oct	15 Nov	7 June 07
	Cleveland, GA	Geneva, NY	13 Oct	13 Oct	17 Nov	6 June 07
		Cleveland, GA	13 Oct	2 Nov	14 Nov	13 June 07
2007	Geneva, NY	Geneva, NY	11 Sept	16 Sept	14 Dec	16 June 08
		Prosser, WA	11 Sept	20 Sept	10 Dec	16 June 08
		Raleigh, NC	11 Sept	3 Oct	10 Dec	16 June 08
		Winchester, VA	11 Sept	3 Oct	10 Dec	17 June 08
	Prosser, WA	Geneva, NY	17 Sept	26 Sept	10 Dec	16 June 08
		Prosser, WA	17 Sept	26 Sept	14 Dec	16 June 08
	Raleigh, NC	Geneva, NY	25 Sept	2 Oct	14 Jan 08	16 June 08
		Raleigh, NC	25 Sept	3 Oct	10 Dec	16 June 08
	Western VA	Geneva, NY	21 Sept	3 Oct	14 Dec	16 June 08
		Winchester, VA	21 Sept	3 Oct	10 Dec	9 June 08
2008	Geneva, NY	Geneva, NY	25 Sept	25 Sept	20 Jan 09	16 June 09

[x] Year listed as the Autumn of the cleistothecia collection.

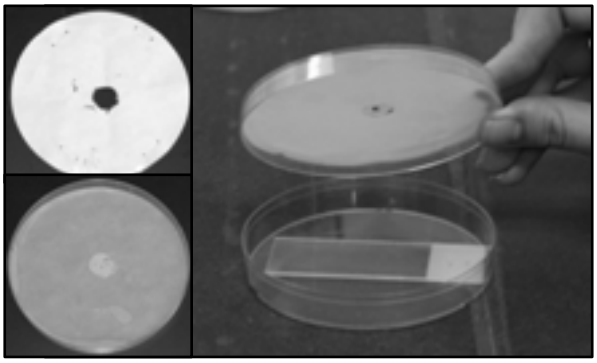

Figure 1.2- Standardized laboratory assay of ascospore discharge. Collected cleistothecia were placed on wet filter paper in closed Petri plates for 24 h at 22-25°C to induce ascospore release over glass slides in the lab.

Comparative maturation and ascospore release of cohorts of ascocarps collected in late summer or early autumn. In 2009, leaves were collected from two separate sites and cultivars: a 30 yr-old *Vitis* interspecific hybrid 'Chancellor' vineyard at Robbins Farm and a 5-year-old *V. vinifera* 'Chardonnay' at Crittenden Farm, both located at NYSAES. Collections were made on 9 Sept ('Wash-Early' collection) and 20 Oct ('Wash-Late' collection) as previously described. Leaves from all nodes on a shoot were collected, and there were 8 rain events over 2.5 mm between collection dates corresponding to potential ascocarp dispersal events (11). Cleistothecia were harvested, prepared and placed on overwintering boards at their respective collection sites, and ascospore discharge tests were performed by taking a small subsample of the cleistothecia on the filter disc and suspending it over a glass slide in a Petri plate as previously described. Additionally, to mimic natural overwintering, cleistothecia were collected as described above to prepare samples that would be overwintered on bark. Cleistothecia suspensions were placed on 2 x 4 cm bark strips by adding a 1 ml drop of the cleistothecia suspension directly to the innermost surface. After air drying, the bark strips were attached to the undersides of vine cordons ('Lower Bark') at their respective sites using plastic-coated twist-ties, with the side bearing cleistothecia

sheltered between the cordon and bark strip (Fig. 1.3). This was also repeated, but by adding the 1 ml cleistothecia suspension directly to the outermost surface of the bark strip, and attaching it, cleistothecia side exposed, on the upperside ('Upper Bark') of the vine cordons.

Figure 1.3- Small bark strips bearing *Erysiphe necator* cleistothecia were attached to both the underside (shown) and upperside (not shown) of vine cordons using plastic-coated twist ties. Bark strips were collected biweekly to weekly in the spring, wetted, and the ability for cleistothecia to release ascospores was tested.

Three replicates samples were collected every two weeks starting in January and every week starting in March. Discharge tests were performed by wetting and affixing the bark strip to the lid of the Petri plate as described earlier for the filter disc release assays, such that the bark surface bearing the cleistothecia faced the glass slide in the bottom of the Petri plate. Ascospore counts were normalized by dividing them by the total number of cleistothecia per replicate bark sample. Average normalized ascospore counts for each sample date were computed, and the cumulative release as a percent of season total was calculated.

Figure 1.4- Placement of filter paper-lined wire-mesh cones in the vineyard for natural trapping of *Erysiphe necator* cleistothecia during autumn rain events. Cones were attached to cordon and cordon trellis wires within the grapevine canopy on both vertical-shoot positioned bilateral cordon trained *Vitis vinifera* 'Chardonnay' at the Crittenden Farm at NYSAES, and sprawling high-wire cordon trained *Vitis* interspecific hybrid 'Chancellor' at the Robbins Farm at NYSAES.

To compare ascocarp maturation and ascospore release in a population of cleistothecia to cohorts collected at discrete times, wire-mesh funnels (5x8 cm; height x diameter) lined with quarter-folded 10 cm filter paper were placed at the above two vineyard locations. Funnels were suspended from cordons with the funnel openings directed upward into the canopy (Fig. 1.4). Sixty nine funnels, spread over 30 vines in 4 vineyard rows, for each of 3 sampling timeframes listed below were deployed at each vineyard site.

Natural rain events dispersed cleistothecia from the foliage (11). Cones were placed in the vineyard for the following time frames (i) 'Early' 9 Sept to 29 Sept 2009,

(ii) ‘Full’ 9 Sept to 20 Oct 2009, and (iii) ‘Late’ 29 Sept to 20 Oct 2009. After deployment, cones were collected from the vineyards and brought into the lab. Filter discs were removed from the cones and affixed to overwintering boards (one per each collection time frame/site combination) as previously described. Boards were then immediately attached to vineyard posts, facing south, in their respective vineyards for overwintering. Starting in January, three filter discs were selected every other week until March, then weekly thereafter. Discharge tests were performed as previously described. Table 1.2 summarizes the 2009 cleistothecia collection and overwintering information. Ascospore counts were normalized by dividing by total cleistothecia per replicate. Average normalized ascospore count for each sample date was computed, and the cumulative release as a percent of season total was calculated.

Weather and phenological data collection. Hourly temperature and precipitation for the NYSAES overwintering site was recorded every 15 min (output averaged over 1 h) by a CR10X datalogger (Campbell Scientific, Inc. Logan, UT) located 6 m from the overwintering boards. Daily temperature and precipitation data were collected from nearby (within 1 km) dataloggers for all overwintering sites. Daily weather data were used to calculate degree day accumulation and precipitation events and used in analysis of the ascospore discharge tests previously described. Differences between the slopes and intercepts of regressed cumulated ascospore release between cohorts at each site were compared using a *t*-test. Specific site information is in Table 1.3.

Table 1.2- Summary of cleistothecia collection types and times for 2009.

Type	Farm[x]	Name	Dates of Collection	Date in Field	First Sample Date	Last Sample Date
Leaf Wash	Rob	Early*	9 Sept	9 Sept	11 Jan 2010	14 Jun 2010
	Rob	Late*	29 Sept	29 Sept	11 Jan 2010	14 Jun 2010
	Rob	Upper	14 Sept	14 Sept	11 Jan 2010	14 Jun 2010
	Rob	Lower	11 Sept	11 Sept	11 Jan 2010	14 Jun 2010
	Crit	Early*	9 Sept	9 Sept	11 Jan 2010	14 Jun 2010
	Crit	Late*	29 Sept	29 Sept	11 Jan 2010	14 Jun 2010
	Crit	Upper	14 Sept	14 Sept	11 Jan 2010	14 Jun 2010
	Crit	Lower	11 Sept	11 Sept	11 Jan 2010	14 Jun 2010
Cone	Rob	Early	9-29 Sept	29 Sept	11 Jan 2010	14 Jun 2010
	Rob	Full	9 Sept- 20 Oct	20 Oct	11 Jan 2010	14 Jun 2010
	Rob	Late	29 Sept- 20 Oct	20 Oct	11 Jan 2010	14 Jun 2010
	Crit	Early	9-29 Sept	29 Sept	11 Jan 2010	14 Jun 2010
	Crit	Full	9 Sept- 20 Oct	20 Oct	11 Jan 2010	14 Jun 2010
	Crit	Late	29 Sept- 20 Oct	20 Oct	11 Jan 2010	14 Jun 2010

* These are labeled as 'Wash-Early' or 'Wash-Late' in the Materials and Methods, and the Results sections.

[x] Research farms at the New York State Agricultural Experiment Station. 'Crit'=Crittenden Farm, 'Rob'=Robbins Farm

Table 1.3- Summary of overwinter locations and websites for associated weather information.

Location	Field/Site Description	Weather Data Website[x]
Geneva, NY	Vineyard at Research Station	http://newa.cornell.edu
Prosser, WA	Vineyard at Research Station (Roza NE)	http://weather.wsu.edu/awn.php
Raleigh, NC	Vineyard at Research Station (Reedy Creek)	www.nc-climate.ncsu.edu/cronos
Chatsworth, NJ	Garden at Research Station (Lake Oswego)	http://climate.rutgers.edu/njwxnet
Cleveland, GA	Lawn at Research Station (Dahlonega)	www.georgiaweather.net/
Winchester, VA	Vineyard at Research Station	*https://apps.cals.vt.edu/cgi-bin/WebObjects/Mesonet.woa/

[x] Website addresses were correct as of 12 May 2010.

* At original time of collection this was an unrestricted service. As of 1 Jan 2010, this was no longer the case.

Figure 1.5- Burkard 7-Day Volumetric Spore Traps were modified to allow for attachment of pieces of grapevine trunk near the intake orifice that were artificially infested with *Erysiphe necator* cleistothecia.

In-field detection of ascospore release. In-field monitoring of ascospore release events was done using a modified Burkard 7-Day Volumetric Spore Trap (Burkard Manufacturing Company, Hertfordshire, England). Modifications consisted of replacement of the existing rain guard with a 4 cm x4 cm guard, along with the addition of a 20-cm aluminum platform positioned directly in line with the orifice as shown in Fig. 1.5. A 20 cm x 3 cm section of grapevine trunk (bark included) was anchored to the aluminum platform and artificially infested with approximately 1000 cleistothecia at a location on the bark sample approximately 4 cm from the intake orifice (Fig. 1.5). Cleistothecia used for infestation were collected on 1 Mar 2010 from filter discs of the 'Wash-Early' and 'Wash-Late' cleistothecia populations at both Crittenden and Robbins Farms.

Airflow of the modified trap was checked using a smoke generator in the laboratory to assure the modified rain shield or sample platform did not change the air stream path near the orifice. Traps were placed in a 6-year-old *V vinifera* 'Chardonnay' plot at the Crittenden Farm at NYSAES on 9 Mar 2010 and were removed on 4 June 2010 (25% bloom). This vineyard developed severe foliar mildew in 2009, produced abundant ascocarps in the autumn, and was selected to maximize the potential background level of ascospore inoculum in the event that the infested bark samples did not provide sufficient propagules for trapping. Temperature, relative humidity, leaf wetness, and liquid precipitation were recorded every 15 min (output was averaged over 1 h) by a CR10X datalogger located approximately 4 m from the traps. Three replicate traps were used, located approximated 3 m apart. Traps sampled 10 L air/min, and the trapping drum clock was adjusted to rotate 2 mm per hour. The Melinex tape in the Burkard trap was replaced every 6 days, and divided into 48-mm (24 h) sections and examined as described by Gadoury and Pearson (1991). Tape sections were mounted in 0.05% Cotton Blue in lactoglycerol, visually inspected for ascospores at 100X under a compound microscope (Leica DMLB, Germany), and ascospores were counted. Ascospore release events were compared to associated weather events (i.e., rain events or periods of continuous leaf wetness within a 24 h period prior to the catching event).

Spread of disease from focal inoculation points established at different grapevine phenological stages. Foci of infection were established at different times in the early growing season in: (i) a *V. vinifera* 'Chardonnay' vineyard at NYSAES (established 2004) in 2008 and 2009, and (ii) a *V. vinifera* 'Riesling' vineyard at NYSAES (established 2005) in 2009. Both vineyards were trained to bilateral cordon, spur pruned and the canopy was trained to vertical shoot positioning. Vines were

planted 2 m apart with 3 m row spacing, with 3 vines per vineyard panel. Vines were hedged at approximately four weeks postbloom.

Disease foci were created by inoculating leaves with 5 µl droplets of a suspension containing 100 conidia/µl in a 0.05% Tween 20 solution. Inoculation points were on the upper and lower surface of the third opened leaf, totaling 4 inoculation points per leaf. One leaf per shoot, two shoots per cordon, and two cordons per disease focus were inoculated, totaling 16 inoculation points. Both cordons of the disease foci were protected during maintenance fungicide applications by covering the shoots with a 1 m x 4 m, 4 mm grade clear plastic sheet, weighted down on each end with a 1.2 m x 2 cm-diameter plumbing grade PVC pipe. These were placed over the vines 1 h prior to fungicide application, and removed within 1 h post application (Fig. 1.6). In addition, the sprayer was turned off while passing over each disease focus.

Inoculation treatments were arranged in a randomized block design, replicated three times in each vineyard (Fig. 1.7, upper) for the 'Chardonnay' trial, and a randomized linear arrangement replicated three times (Fig. 1.7, lower) for the 'Riesling' trial. In 2008, blocks (single treatment) in the 'Chardonnay' vineyards were 2 panels long (6 vines) and five rows wide. In 2009, blocks in the 'Chardonnay' vineyard were 2 panels long and 3 rows wide. Inoculation hotspots were located on adjacent cordons of the third and fourth vine of the middle row in each block. Treatments consisted of three different inoculation time points and an uninoculated control. Blocks in the 'Riesling' vineyard were 1 panel long and 3 rows wide. Inoculation hotspots were located on both cordons of the second vine in the middle row of each block. Single buffer panels that received full-season spray programs were located between each replication block. Panels in both blocks consisted of 3 vines between trellising support poles. Rows were continuous linear panels (Fig. 1.7).

Treatments consisted of three different artificial infection time points and an uninoculated control. Vines were inoculated with *E. necator* conidia at Eichhorn and Lorenz (5) grapevine development stages (i) EL12 (10 cm shoots, 5 separated leaves), (ii) EL17 (inflorescence visible, 12 separated leaves), or (iii) EL23 (50% cap fall, full bloom). In 2008, these dates were 26 May, 5 June and 13 June, respectively. In 2009, these dates were 19 May, 1 June and 21 June, respectively.

Fungicides were applied between 6 and 9 a.m. using a hooded-boom, single-row sprayer. Dates of fungicide applications to control endemic powdery mildew are listed in Table 1.4.

Figure 1.6- Inoculated foci of grape powdery mildew were protected from summer fungicide sprays using plastic sheets weighted down with PVC pipe. Sheets were placed over the disease foci directly before spraying, and were removed within 1 h of the spray. To reduce fungicide drift underneath the sheets, the spray was turned off when passing over each focus.

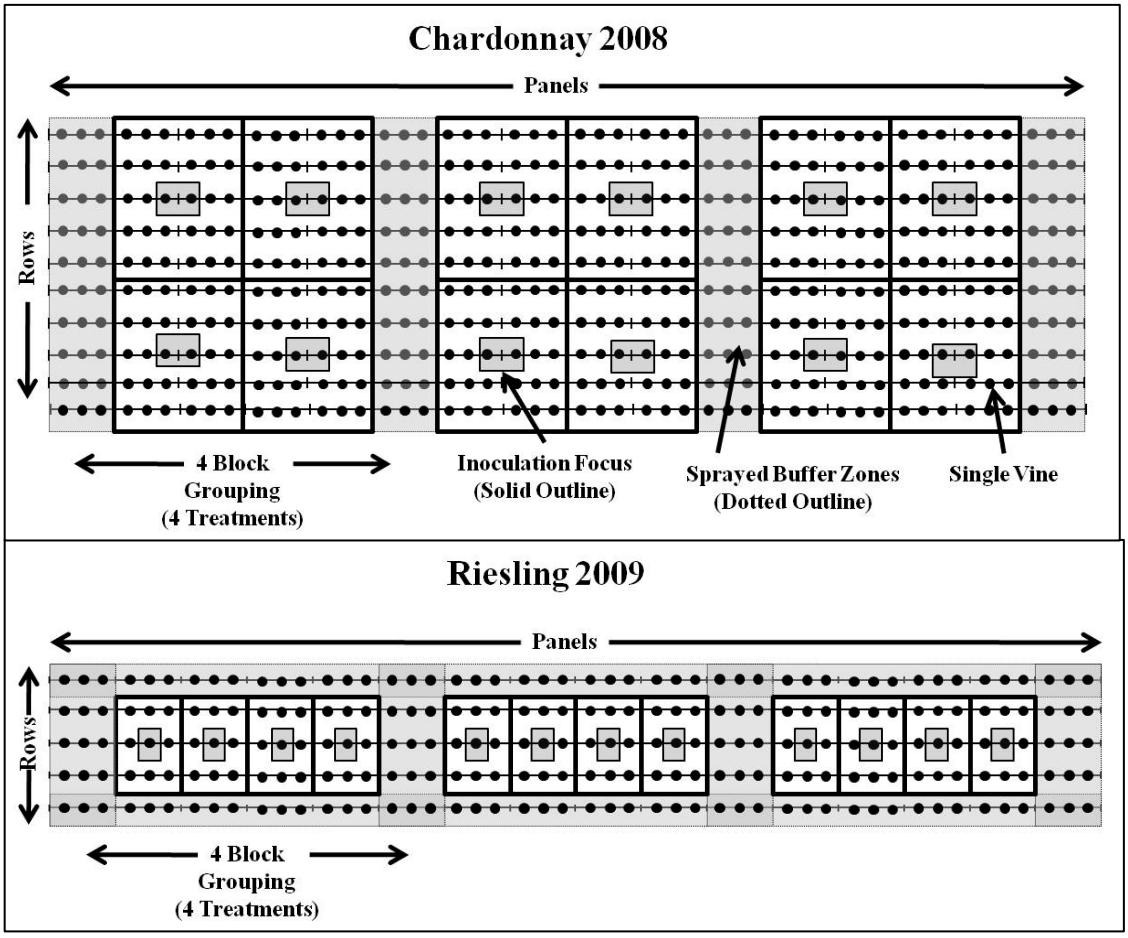

Figure 1.7- Schematic of vineyard experimental design for the 2008 *Vitis vinifera* 'Chardonnay' and 2009 *V. vinifera* 'Riesling' disease foci experiments. Each row consisted of 16 continuous panels and each panel had 3 vines. A block consisted of a single treatment.

Table 1.4- Spray dates for 2008 and 2009 on both the *Vitis vinifera* 'Chardonnay' and *V. vinifera* 'Riesling' plots used to establish powdery mildew disease foci. Clusters serving as bio-indicators were protected from sprays using food-grade plastic bags.

Date	Material	Date	Material
30 May 2008	quinoxyfen	29 May 2009	sulfur
	sulfur		
9 June 2008	quinoxyfen	9 June 2009	sulfur
	sulfur		pyraclastrobin+boscalid
23 June 2008	quinoxyfen	22 June 2009	quinoxyfen
	sulfur		sulfur
7 July 2008	quinoxyfen	2 July 2009	quinoxyfen
	sulfur		
18 July 2008	quinoxyfen	17 July 2009	quinoxyfen
	fenarimol		
1 Aug 2008	quinoxyfen	4 Aug 2009	sulfur
	sulfur		pyraclastrobin+boscalid

Clusters served as bio-indicators for the spread of powdery mildew conidia and were protected from sprays by a 1 liter food-grade plastic bag placed over each cluster approximately 1 h prior to spraying, and removed within 1 h postspray. The designated disease foci were only protected from fungicide applications 1 week prior to, and all season after, inoculation with *E. necator* conidia. Otherwise, disease foci received fungicide treatments to reduce unintentional powdery mildew establishment. Whole clusters were harvested and rated for powdery mildew incidence (number of infected berries/cluster) and severity (surface area of cluster infected) on 10 Aug 2008, and on 1 Sept 2009. Early harvest in 2008 was due to high incidence of Botrytis bunch rot.

Severity (percent surface area infected) of powdery mildew infection on clusters (40 clusters/ treatment in 2008; 24 clusters/treatment in 2009) throughout the plots for each inoculation treatment was regressed against physical linear distance from the inoculation focus. Qualitative cluster severity contour plots were constructed

to visualize foci effects by plotting disease severity versus coordinates delineating the position of a rated cluster according to its location within each treatment block. Maps were based on the average severity of pooled replicates for each treatment.

Statistical Analyses. Unless otherwise indicated, all statistical analyses were performed using JPM Statistical Software (SAS Institute, Inc., Cary, North Carolina, USA).

RESULTS

Cleistothecia collection, overwintering, and discharge assessments. *Effects of overwintering location on ascospore release.* The cumulative seasonal total ascospore release, from 8 to 90%, of the NY populations overwintered in NY was regressed against the number of putative discharge events (rain> 2.5 mm coincident with maximum daily temperatures above 0°C from 1 January), and yielded a model where R^2=0.74, and b_0 (intercept) was not significantly different than 0 (-6.93, P=0.25) and b_1 (slope) was significantly different from 0, (3.17, P<0.0001). The 95% confidence bands indicate that 25% of the season total ascospore release for NY populations overwintered in NY occurred between 5 and 12 ascospore release events; 50% occurred between 16 and 20 ascospore release events; and 75% occurred between 23 and 30 ascospore release events. Regression of combined populations of cleistothecia (NC, GA, WA, NJ, and NY) overwintered in NY, resulted in a model where R^2=0.69, and b_0 was different than 0 (-11.13, P=0.03) and b_1 was significantly different from 0, (3.58, P<0.0001). In 2006, 2007, 2008 and 2009, the date of 50% season total ascospore release occurred on climate day of year (CDOY; calendar numbering starting 1 Mar) 26, 47, 24, and 59, respectively, and *V. vinifera* 'Chardonnay' budbreak occurred on CDOY 68, 71, 59, and 50, respectively.

Regression analysis of ascospore release for both WA and NY populations overwintered in WA to total ascospore events resulted in two distinct data groupings: seasonal total ascospore release occurred over very few putative release events in 2006 and 2007 (less than 7), and occurred over a larger number a putative release events in 2008 (greater than 15). However, when season total ascospore release was regressed against to the combination of ascospore release events as described above and degree day accumulation (base 0°C starting 1 Jan), these two distinct groups converged. The resultant regression had an R^2=0.76, b_0 was not significantly different than 0 (-3.45, P=0.61) and parameter estimates were significant for both ascospore release events (1.19, P=0.0002) and degree day accumulation (0.08, P<0.0001). In 2006, 2007, and 2008, the date of 50% season total ascospore release occurred on CDOY 52, 47,and 60, respectively, and *V. vinifera* 'Chardonnay' budbreak occurred on CDOY 55, 54,and 75, respectively.

Regression analysis of both NJ and NY populations that overwintered in NJ showed that the dominant factor for ascospore release in this location was not the number of ascospore release events, but was a simply a factor of degree day accumulation. When combined with ascospore release events, the regression resulted in a model with R^2= 0.59, b_0 not significantly different than 0 (8.09, P=0.24), an insignificant parameter estimate for ascospore release events (0.14, P=0.94) and a significant parameter estimate for degree day accumulation (0.12, P<0.03). In 2006 and 2007, the date of 50% season total ascospore release occurred on CDOY -4 and 41, respectively, and *V. vinifera* 'Cabernet franc' budbreak was estimated to occur on CDOY 51 and 52, respectively. Estimated New Jersey budbreak dates were based on VA dates (similar USDA Climate zones) as NJ phenology data was not recorded.

The time of ascospore release of NY populations of cleistothecia overwintered in VA could also be explained by accumulating putative ascospore discharge events as

defined above. Regression resulted in a model R^2=0.80, b_0 not significantly different than 0 (1.01, P=0.33) and b_1 significantly different from 0 (4.44, P<0.0001). The 95% confidence bands around the fitted line indicated that 25% of the season total ascospore release occurred between 0 and 5 ascospore release events; 50% release occurred between 7 and 12 release events, and 75% release occurred between 15 and 20 release events. In 2007, and 2008, the date of 50% season total ascospore release occurred on CDOY 50 and 10, respectively, and *V. vinifera* 'Chardonnay' budbreak occurred on CDOY 51 and 52, respectively.

The time of ascospore release of NY and NC populations of cleistothecia overwintered in NC could also be explained by accumulating ascospore events as defined above. Regression analysis resulted in a model R^2=0.60, and b_0 and b_1 significantly different from 0 (18.1, P=0.007; and 7.35, P<0.0001, respectively). The 95% confidence bands around the fitted line indicate that 25% of the season total ascospore release occurred between 0 and 2 ascospore release events; 50% release occurred between 4 and 6 release events, and 75% release occurred between 7 and 12 release events. In 2006, 2007, and 2008, the date of 50% season total ascospore release occurred on CDOY 22, 27 and 22, respectively, and *V. vinifera* 'Chardonnay' budbreak occurred on CDOY 32, 57,and 32, respectively.

When all populations and locations were combined and ascospore release was compared to the number of ascospore release events and degree day from 1 Jan, the regression model had an R^2=0.53. The model was a significantly better fit than the mean response (P<0.0001), and slopes for both degree day and ascospore release events were significantly different than 1 (slope coefficients of 1.53, P<0.0001, and 0.06, P<0.0001, respectively).

Low numbers of cleistothecia in samples from VA populations overwintered in VA and NY, and severe microbial degradation of both NY and GA cleistothecia

populations overwintered in GA prevented analyses of these particular population:site combinations.

Comparative maturation and ascospore release of cohorts of ascocarps collected in late summer or early autumn. Different cohorts of cleistothecia from the controlled 'Wash' collections did not differ in their times of first and final laboratory-induced ascospore release event. However, ascospore accumulation curves were different (Fig. 1.8). Cleistothecia collected at different times using the "Wash" method from 'Chancellor' vines at Robbins Farm had a difference in the point of 50% ascospore release of 21 d (Fig 1.8A). Similar results were seen for cleistothecia cohorts from 'Chardonnay' vines at Crittenden Farm, where the point of 50% season total ascospore release was separated by 28 d, but the variance in the release curves for the 'Wash-Early' and 'Wash-Late' populations made this separation less distinct (Fig. 1.8B). Regression of 10-95% ascospore release against CDOY resulted in the following models: for Crittenden Farm 'Wash-Early', had an R^2=0.57, and b_0 and b_1 were different from 0 (60.53, P<0.0001; and 0.80, P=0.004, respectively). The 'Wash-Late' population had an R^2=0.62, where b_0 was not different from 0 (13.59, P=0.08) but b_1 was (1.03, P<0.0001). Robbins Farm 'Wash-Early' had an R^2=0.82, and b_0 and b_1 were different from 0 (51.93, P<0.0001; and 0.71, P<0.0001, respectively). The 'Wash-Late' population had an R^2=0.88, and b_0 and b_1 were different from 0 (10.52, P=0.001; and 1.01, P<0.0001, respectively). While the slopes between the 'Wash-Early' and 'Wash-Late' cohorts for Crittenden Farm were not statistically different (P=0.36), the intercepts were (P<0.0001). Both the b_1 and b_0 were different for each other for the two cohorts at Robbins Farm (P=0.002 and P<0.0001, respectively). Different intercepts confirm the shift in peak ascospore release between the two cohorts.

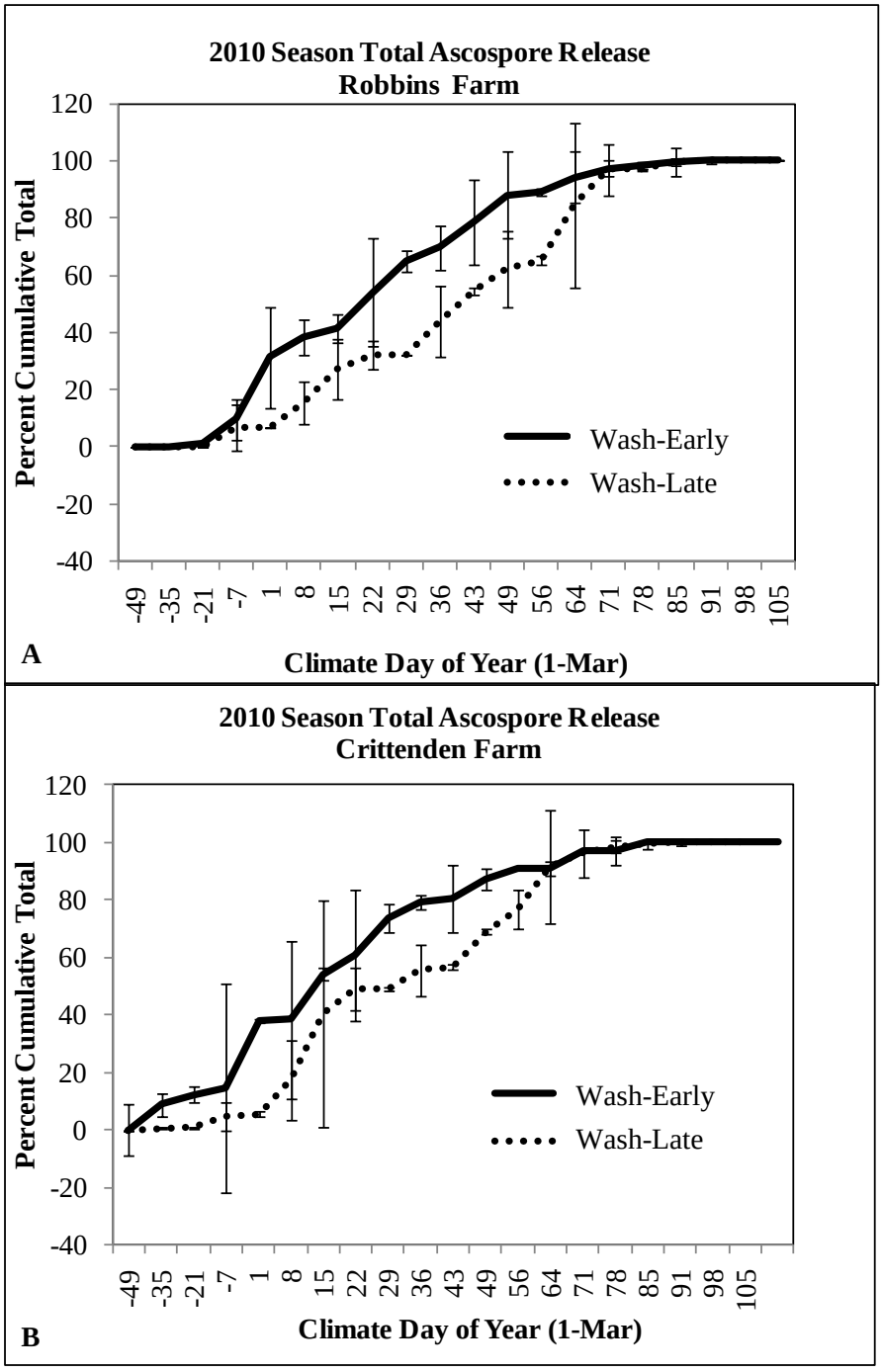

Figure 1.8- 'Wash-Early' and 'Wash-Late' *Erysiphe necator* cleistothecia populations from 2010 have similar start and end points in the distribution, but peak ascospore release is offset. There was a more distict seperation in release for populations at A) Robbins Farm compared to, B) Crittenden Farm.

Populations of cleistothecia collected from natural rain events had lower total cleistothecia numbers at the time of discharge tests compared to collections done via the 'Wash' method described above (an average range of 20-80 versus 80-172 cleistothecia/subsample for the naturally collected versus the 'Wash' populations). At the Crittenden Farm, only 'Full' season mesh cone collection had sufficient cleistothecia counts to permit proper analysis with rain events.

Ascospores were only detected on one to two test dates for the 'Early' (CDOY 49 and 64) and 'Late' (CDOY 64) cohorts, respectively. Samples from Robbins Farm had enough cleistothecia and successful discharge tests to produce ascospore accumulation curves, as seen in Fig 1.9. Similar to the 'Wash' populations, the 'Early' and 'Late' populations appear to be separated by 14-21 d, but the start and end of ascospore release were not affected by time of collection.

Few ascospores were trapped from cleistothecia populations overwintered on bark strips at Crittenden Farm (totaling 6 ascospores over the course of all sample periods, from two separate dates and from 'Lower Bark' only). A total of 54 ascospores were trapped from cleistothecia populations overwintered at Robbins Farm, from 25 Jan 2010 to 10 May 2010, a timeframe similar to that of populations overwintered on filter discs. Forty-two percent of the total spores originated from 'Upper Bark' samples and 58% were from 'Lower Bark' samples. Of the season total, 40 ascospores were released on a single sample date, 25 Apr, of which 55% of the total spore number came from 'Upper Bark' samples, and 45% came from 'Lower bark samples. Total remaining cleistothecia at the time of discharge tests were low, and ranged from an average of 5-36 cleistothecia on 'Upper Bark' samples at Robbins Farm, to 13 to 353 cleistothecia on 'Lower Bark' at Robbins Farm. Crittenden populations had an average of 6-26 cleistothecia on 'Upper Bark' samples, and 23-116 cleistothecia on 'Lower Bark' samples.

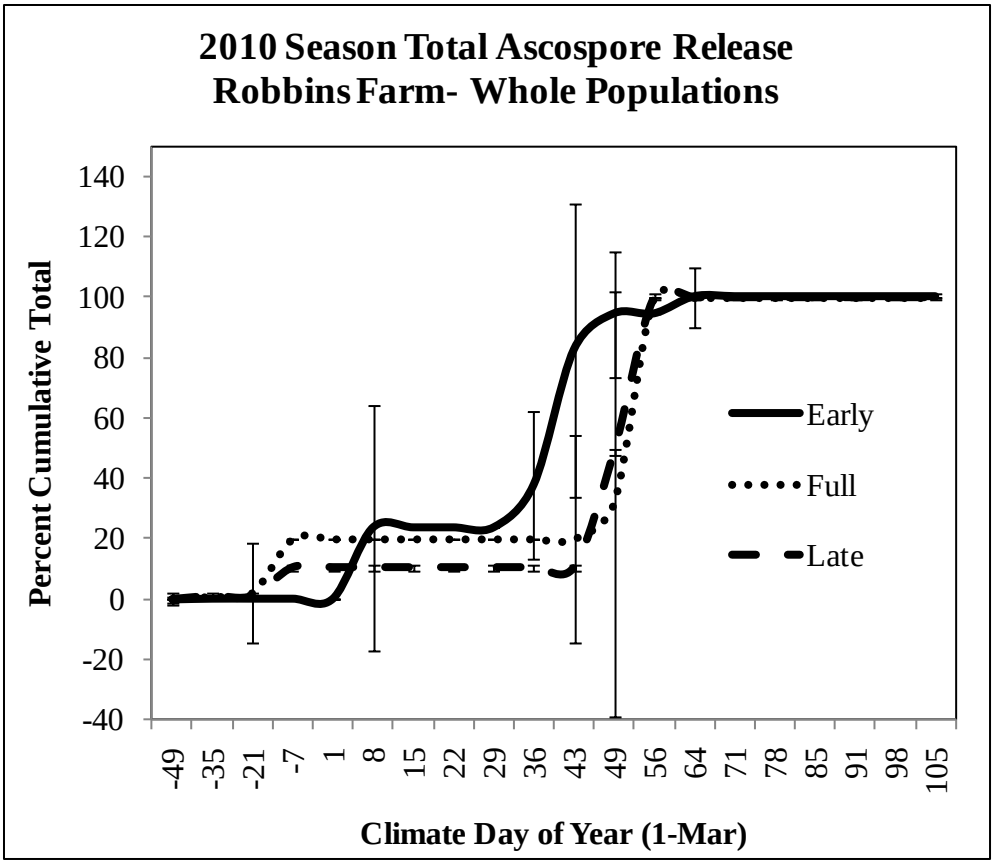

Figure 1.9- *Erysiphe necator* ascospore release curves from naturally collected cleistothecia cohorts on the *Vitis* interspecific hybrid 'Chancellor' at Robbins Farm in 2010. 'Early', 'Full', and 'Late' collection occurred from 9-29 Sept, 9 Sept-20 Oct, and 29 Sept-20 Oct 2009, respectively.

In-field detection of ascospore release. Ascospore capture was at the threshold level of detection for the Burkard Volumetric Spore Traps, despite the concentrated deposit of cleistothecia and proximity to the intake orifice. After the cessation of experiments, the artificially-inoculated grapevine trunk sections were inspected, and no remaining cleistothecia could be located.

Most ascospores trapped were associated with either 3+ hours of continuous leaf wetness, or rain events that occurred within the previous 24 h (12 of 17 events). There were instances, however, when trap events were not associated with moisture. Table 1.5 contains a summary of the dates, associated weather data, and total

ascospores trapped during each event from March to June 2010. This time frame also coincided with ascospore release from populations overwintered on filter discs.

Table 1.5- Summary of total number of *Erysiphe necator* ascospores caught by the Burkard Volumetric Spore Trap from March to June 2010.

			24 h prior		Total Ascospores
Date	**CDOY**	**Time**	**Rain (mm)**	**Temp (C, Ave)**	
x:15-Mar	12	12-Mar	1.90	8.20	1
	13	13-Mar	21.00	5.30	
	14	14-Mar	18.00	4.00	
	15	15-Mar	5.80	5.80	
27-Mar	27	12:30	3 h**	-4.40	1
28-Mar	28	9:00	0.00	2.70	1
3-Apr	34	3:30	0.00	19.90	1
3-Apr	34	6:00	0.00	20.20	1
3-Apr	34	23:30	0.00	19.70	1
4-Apr	35	9:45	0.00	18.45	1
8-Apr	39	1:00	1.93	18.30	1
26-Apr	57	13:30	24.51	10.20	1
28-Apr	59	5:00	1.93	4.40	3
1-May	62	9:00	3 h**	17.70	4
2-May	63	2:00	6 h**	20.20	1
5-May	66	6:00	7.09	14.80	1
5-May	66	23:00	19.35	17.43	1
8-May	69	2:00	27.09	10.66	1
12-May	73	23:00	19.99	6.40	1
18-May	79	10:00	7 h**	15.61	1

* Spore trap clock quit functioning during the trapping timeframe, therefore the official start time for that section of catching tape could not be determined

** Continuous hours of leaf wetness within the previous 24 h of spore trapping.

Of the 15 rain events (>1.0 mm) in April and May, 8 were associated with a detectable ascospore release event. Seven of these rain events were concentrated between 25 Apr and 11 May. Some ascospore release events were not associated with

any identifiable weather event: One event was cool with light rain (28 Apr), and the other was warm with only a short period of continuous leaf wetness (1 May). From 12 Mar to 6 June, the spore traps were exposed to a total of 25 rain events (>1.0 mm).

Spread of disease from focal inoculation points established at different grapevine phenological stages. Powdery mildew developed in the inoculated foci in the both the *V. vinifera* 'Chardonnay' experiment in 2008 and 2009, and in the *V. vinifera* 'Riesling' experiment in 2009. The combination of favorable conditions for powdery mildew development and the high level of background inoculum carry-over resulted in all treatments, including the control, which reached 100% incidence and high levels of cluster disease severity values in the 2009 'Chardonnay' experiment. Therefore, this trial was not used in further analyses. The uninoculated control treatment of the 2009 'Riesling' experiment had high disease severity in the focus location due to background inoculum levels and lack of maintenance fungicides due to protection from sheeting as described in the Materials and Methods. In both years, clusters within 0.5 linear meters of the inoculated foci for EL12, EL17 and EL23 had reached maximum disease severity by the end of the July.

Disease severity on clusters was significantly inversely related to distance (m) from the inoculation foci in the 2008 'Chardonnay' EL12, EL17, and EL23 treatments, with slope coefficients of 70.7 ($P<0.0001$), 36.2 ($P<0.0001$), and 20.18 ($P<0.0001$), respectively. There was not a significant inverse relationship between distance from focus and disease severity for the control 3.53 ($P=0.40$). Intercepts were not significant for the EL12 and EL 17 treatments (-2.58, $P=0.22$, and 2.12, $P=0.21$, respectively), but were significant for the EL23 and control treatments (5.58, $P=0.007$, and 9.15, $P<0.0001$, respectively). In the 2008 'Chardonnay' experiments, the maximum cluster severity (>75%) occurred within 1 m of the inoculated disease foci for all treatments except the control, which did not reach this level of severity.

Disease severity on clusters was significantly and inversely related to distance (m) in all treatments in the 2009 ‘Riesling’ experiment, with slope coefficients of 55.2 (P<0.001), 19.2 (P=0.003), 38.1 (P<0.001), and 41.1 (P<0.001) for the EL12, EL17, EL23 and control treatments, respectively. Intercepts were not significant for any of the 2009 treatments (-4.03, P=0.42; 7.82, P=0.07; 2.15, P=0.68; and 0.65, P=0.90, respectively). For the 2009 ‘Riesling’ experiment, the maximum cluster disease severity (>75%) occurred within 3.5 m of the inoculated disease foci for all inoculated treatments and the focus location in the uninoculated control treatment. Severity levels >90% occurred within 1.5 m for all treatments except the EL17.

Qualitative contour maps of cluster disease severity spatial distributions for both the 2008 ‘Chardonnay’ and 2009 ‘Riesling’ experiments are seen in Fig. 1.10.

There was a high incidence of Botrytis bunch rot in 2008 across all treatments, without a treatment effect (P<0.05, Student’s t-test). There was a significant negative linear relationship between distance in from the ‘hotspot’ of the EL12 treatment and Botrytis bunch rot severity (slope coefficient of –15.77, P=0.0006). The remaining treatments, EL17, EL23 and the control did not have a significant relationship between Botrytis bunch rot severity and distance from the inoculated ‘hotspots’.

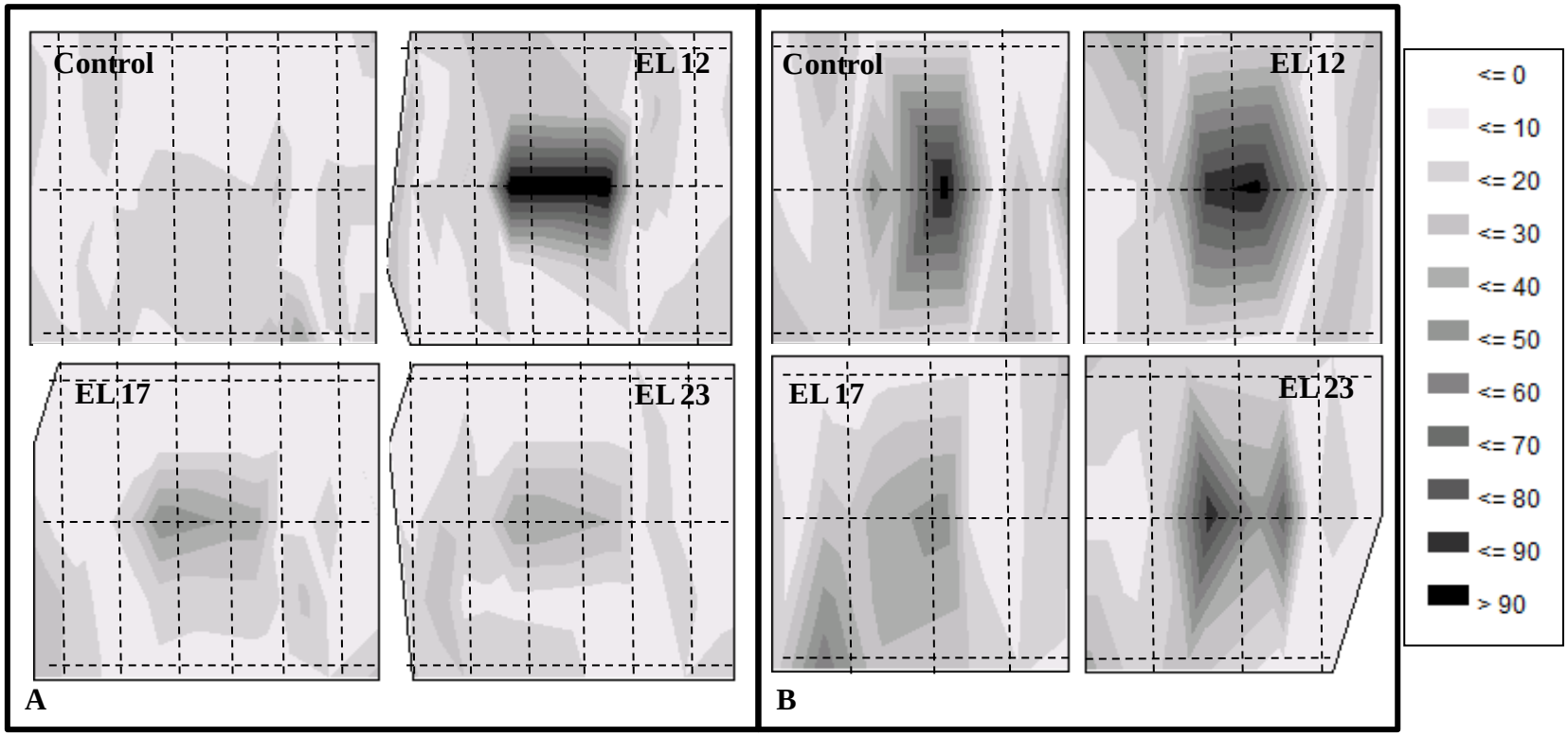

Figure 1.10- Contour plots of powdery mildew cluster severity (% total surface area, legend on the right) resulting from disease foci for A) *Vitis vinifera* 'Chardonnay' in 2008 and B) *V. vinifera* 'Riesling' in 2009. Inoculation time points were: EL12 (10 cm shoots, 5 separated leaves), EL17 (inflorescence visible, 12 separated leaves), and EL23 (50% cap fall, full bloom). Controls were uninoculated and unsprayed, and were evidence of background levels of inoculum in the vineyard plot. Intersection of dotted lines indicates individual vine location, which were planted 1 m apart within the row. Rows are indicated by horizontal dotted lines.

DISCUSSION

Previous field-based ascospore capture methods (12, 23, 27, 31) rarely found ascospore release in the months preceding local *V. vinifera* budbreak, and as a consequence, existing powdery mildew models do not assess the impact of prebudbreak weather conditions on inoculum survival (1, 24). Data presented here show that cleistothecia are capable of discharging ascospores long before budbreak; by the time local *V. vinifera* phenology had reached budbreak, 50% of the seasonal total of ascospores in 85% of the site/year combinations had been released. This raises the question of why an obligate biotroph would release progeny in advance of available host tissue, as it would seem to be an ill-fated evolutionary move. However, powdery mildews are typical r strategists in ecological development, relying on the production of a large number of offspring to ensure genetic propagation and reproduction success rather than careful care of few genetic offspring. Thus, for grapevine powdery mildew, accurate alignment with *V. vinifera* budbreak may not be as crucial to progeny survival as the simple ability to produce high numbers of cleistothecia. The accuracy of ascospore release as aligned to grapevine phenology is improved, however, if it is compared to phenology of the indigenous *V. riparia* in upstate New York, as vine development is generally 2-3 weeks in advance of the introduced *V. vinifera* species.

Overwintering conditions are what drives the timing of ascospore release in cooler-climates. Release is dependent upon the number of wetting events and temperature in late winter/early spring. Wetting events, or liquid precipitation greater than 2.5 mm, are necessary for cleistothecia dehiscence (12), and the time of grapevine phenology is related to late winter/spring heat unit accumulation (17, 28). Unfortunately, attempts to draw a generalization to predict ascospore release across a variety of climates was a challenge. This challenge may be a product of the experimental design: inconsistency in collection of cleistothecia populations due to

geographical limitations; inconsistency in overwintering sites; potential problems with sample delivery; and general site limitations as seen in VA and GA where warm overwintering conditions led to high parasitism of cleistothecia. Additionally, in warmer climates, a higher number of potential pre-season rain events and an increased rate of degree day accumulation could result in early inoculum depletion and may be a reflection of threshold overload, and not a true reflection of a release response timed for evolutionary survival.

There is also a question of whether the experimental design for artificially overwintered cleistothecia could induce differences in discharge. However, other studies have also used similar methods in collecting and overwinter cleistothecia (1). In addition our corresponding bark and Burkard tests, which served as controls to the other overwintering tests, had similar start and end points of ascospore release when compared to samples overwintered on filter discs. This indicates that the experimental design was not a likely cause for the shift in ascospore release.

Studies in 2010 that compared ascospore release from different cohorts of cleistothecia showed that in New York, the time of cleistothecia maturation in the autumn does influence the time of peak ascospore release the following spring, but it does not appear to influence the time of initial and final release from controlled collections with high numbers of cleistothecia. Reports on ascospore release in warmer climates have shown a period of two ascospore release seasons: one in the autumn and one in the spring (22). This phenomenon may be related to the ability of early-formed cleistothecia to accumulate the necessary heat units to reach release maturity well before grapevine leaf senescence. This might also explain the differences in peak ascospore release observed in the New York cohorts: those collected and matured early could accumulate more autumn heat units than those collected and matured later in the autumn. While the start and end of the ascospore

release curves were similar between the early and late collection timeframes, early collections provided between 51 and 60% of the season total ascospore release by 1 March (Crittenden and Robbins Farms, respectively), whereas the late collections provided only 10 to 13% release by this same time. The presence of a cohort difference in ascospore release could influence epidemic development the following year, if environmental and weather events happen to favor the survival of one particular cohort over another. Late-summer heavy rains (11) or an early frost which would induce leaf senescence would favor the selection an early cohort of cleistothecia potentially resulting in an earlier distribution of ascospore release the following spring. This could result in an exhaustion of primary inoculum before the availability of susceptible host tissue, thus either halting or delaying epidemic development during the growing season.

Conversely, autumn rains and protracted warm temperatures would favor the dispersal and survival of the entire population of cleistothecia consisting of multiple cohorts, leading to a very wide distribution timeframe of ascospore release the following year. Intense foliar disease management through the growing season would delay the development of cleistothecia (18, 35), and thus favor a late-season cohort, potentially delaying the distribution of ascospore release the following year. While this delay in development of cleistothecia may favor the alignment of ascospore release to grapevine phenology the following spring, it reduces the total cleistothecia numbers that would serve as a primary inoculum source the following season.

The differences seen in ascospore release distribution, whether related to overwintering conditions or to previous fall development (cohorts), may help explain the boom and bust cycles of powdery mildew disease severity on clusters seen in New York State. Combinations of early cleistothecia cohort selection and significant late winter/early spring rain events would favor early ascospore release and potential

inoculum exhaustion, while warmer and extended late summers/autumn, and dryer, cooler late winter/early springs would more closely align ascospore release to the availability of susceptible host tissue.

Timing of primary inoculum arrival can significantly influence epidemic development, all else being equal. However, data presented here also suggest that the overall favorability of the in-season weather is really what determines the extent and rate of epidemic development from an infection site, and this was also seen in other studies on the influence of primary inoculum on cluster infection (27). In years where the environment is not as favorable for powdery mildew (2008), early establishment may be essential for severe fruit infection. In years where the environment is extremely conducive for powdery mildew development, the time of inoculum arrival is less important, as there is sufficient time for primary infections to establish and spread while the fruit is susceptible. Even with favorable weather, powdery mildew disease severity gradients are steep from infection foci, which indicates that wide-spread severity in a vineyard may be a result of multiple, simultaneous primary infections. In New York State, this would be the result of wide-spread cleistothecia production and distribution onto grapevine cordons and trunks the previous year (11, 31).

In the 2009 *V. vinifera* 'Riesling' experiments, all but the EL17 inoculation time point (including the control), had high levels of powdery mildew disease severity on grape clusters. A possible explanation for this is that the designated "hotspots" in the EL17 and EL23 treatments in the 2009 'Riesling' experiment did receive an initial first fungicide spray to reduce unintended infections, where the control did not. However, shortly after the EL17 inoculation, a cold event occurred in the vineyards, potentially debilitating colony development (see Chapter 2 for further detail). The EL23 inoculation occurred at a time that when environmental conditions were

favorable for mildew establishment and secondary development, which is why there was a larger gradient of disease spread and higher cluster severity compared to the 2008 EL23 *V. vinifera* 'Chardonnay' trial.

In conclusion, ascospore release and subsequent initiation of grapevine powdery mildew epidemics may be early compared to local *V. vinifera* phenology, but spans a wide time frame which encompasses budbreak for other local species of *Vitis* such as *riparia* and *labrusca*. The time of ascospore release is related to the number of wetting events and temperature accumulation in the late winter/early spring. In contrast to previous studies, however, ascospore release appears to occur much earlier than initially described in New York State. This may suggest that severe cluster infections are not the result of direct ascosporic infection as is often inferred, but a result of earlier establishment and subsequent build-up of secondary inoculum as a result of favorable in-season weather conditions.

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CHAPTER TWO

EFFECTS OF ACUTE LOW TEMPERATURE EVENTS ON DEVELOPMENT OF *ERYSIPHE NECATOR* AND SUSCEPTIBILITY OF *VITIS VINIFERA**

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ABSTRACT

Growth and development of *Erysiphe necator* (syn. *Uncinula necator*) has been extensively studied under controlled conditions, primarily with a focus on development of grapevine powdery mildew within the optimal temperature range and the lethal effects of high temperatures. However, little is known of the effect of cold temperatures (above freezing but less than 8°C) on pathogen development or host resistance. Pretreatment of susceptible *Vitis vinifera* leaf tissue by exposure to cold temperatures (2 to ≤8ºC for 2 to 8 h) reduced infection efficiency and colony expansion when tissues were subsequently inoculated. Furthermore, nascent colonies exposed to similar cold events exhibited hyphal mortality, reduced expansion, and increased latent periods. Historical weather data and an analysis of the radiational cooling of leaf tissues in the field indicated that early-season cold events capable of inducing the foregoing responses occur commonly and frequently across many, if not most viticultural regions worldwide. These phenomena may partially explain: (i) the unexpectedly slow development of powdery mildew during the first month after budbreak in some regions, and (ii) the sudden increase in epidemic development once seasonal temperatures increase above the threshold for acute cold events.

Additional Key Words: induced resistance, acute cold exposure, stress physiology

INTRODUCTION

Powdery mildews occupy a distinct ecological and biological niche. Among the plant pathogenic fungi, they are unique in that, with the exception of the haustoria in parasitized epidermal cells, the preponderance of their biomass is external to the host. This uniquely external and exposed condition makes them especially responsive to a variety of environmental factors, including temperature (4, 7, 37), solar radiation (2), and atmospheric humidity (7). A number of simple weather-driven models designed to predict powdery mildew infection risk and general epidemic development have been developed from reanalysis of data generated in laboratory and greenhouse studies of the disease (24, 29, 36). However, such models focus on epidemic development when conditions are in the optimal growth range for the pathogen, with particular emphasis on regional weather phenomena that alter epidemic development. There is close agreement among several laboratory and controlled environment studies with respect to the cardinal temperatures defined for spore germination and infection of host tissues by *Erysiphe necator* (4, 7, 37). Simple regression models based on germination, infection, or colony expansion data generated at various controlled temperatures are the basis of some of the most widely used advisory systems for timing of fungicide applications (24, 29). However, deployment of the advisory system often requires an empirical approach to adjust model outputs to provide a better fit to observed disease development in vineyards. Some of these output adjustments, in particular those relating to suboptimal temperatures, cannot be easily reconciled to the effects observed in laboratory studies. For example, Delp (7) demonstrated that germination of conidia on grapevine leaves at 17°C was reduced by only 29%, and subsequent infection was reduced by 50%, compared to the optimum of 26°C. Similar results were reported for ascospore germination and infection (14). However, some advisory systems rate the risk of infection as low at temperatures

below 15 to 19°C (5, 24, 29). Additionally, disease development in the field has been investigated using ambient air temperature, which can differ substantially from leaf surface temperatures in field environments. Leaf surface temperature in direct sun can exceed ambient air temperature by several degrees (2), and at night ambient air temperature can exceed leaf surface temperature by several degrees due to radiational cooling of leaf tissues (40).

These disparities between (i) the effects of temperature on disease development in model advisories versus lab studies, and on ambient air versus leaf surface temperature, combined with (ii) the unexpectedly slow development of powdery mildew epidemics compared to model forecasts that we have observed locally, led us to initiate a number of biological and epidemiological studies on the effects of acute, cold temperature events (above freezing but less than 8°C) on grape tissue response to powdery mildew infection and powdery mildew development (17, 30-32). We consistently obtained responses that were similar to those observed in leaves transitioning to or in an ontogenically resistant state, i.e., quantitatively reduced infection efficiency, increased latent period, and increased mortality of hyphae in the colony (32). In the present study, we investigate the impact of acute cold events on the induction of a resistance response in susceptible *Vitis vinifera* leaf tissues and upon the development of nascent mildew colonies under both controlled conditions and in the field. We also show that acute cold events are not solely a feature of cold climates, but are common features early in the growing season in several warm-climate viticultural regions.

MATERIALS AND METHODS

Grapevine cultivar selection and susceptibility to powdery mildew. Three cultivars of a highly susceptible grapevine species, *V. vinifera* (14), were used in

experiments. Leaves of *V. vinifera* cvs. 'Chardonnay', 'Riesling', 'Pinot noir' and 'Cabernet franc' reportedly have similar susceptibility to powdery mildew infection (34). Seedlings grown from open-pollinated 'Chardonnay' vines were used for inoculum propagation and pathogenicity tests (10, 14, 18).

Preinoculation exposure of grapevine leaves to acute cold events. Seedlings from stratified seed harvested from open-pollinated *V. vinifera* 'Chardonnay' were grown in pots at 22°C with a 14 h photoperiod illuminated by daylight balanced fluorescent bulbs (5500 K). After 6 weeks of growth, the most ontogenically susceptible leaf which is generally the second or third leaf below the shoot apex, about 50 to 75% expanded, and with a noticeably more shiny cuticle than older leaves (9), was detached and surface disinfested in 0.5% $NaOCl_2$ for 90 s, rinsed twice in distilled water and incubated overnight at 22°C on 1% water agar plates, adaxial surface upright with petiole inserted into the agar. Cold temperature treatment levels and durations of each temperature treatment for each experiment are described in the following text. All treatments occurred in the dark and leaves were returned to 22°C posttreatment.

Inoculum preparation. Conidial inoculum of *E. necator* was cultured on detached seedling leaves on 1% water agar at 22°C with 14 h photoperiod as mentioned above. Colonies were transferred to new leaves every 14 days to maintain their vigor and ensure conidia germinated when transferred to host tissue. Ten-day-old colonies were used as an inoculum source for all experiments. Germination potential of conidia was assessed immediately before and after each inoculation by transferring a sample of the inoculum to a glass microscope slide. The slide was incubated in a closed Petri dish with moist filter paper for 24 h at 22°C, after which the percent germination (i.e., presence of a germ tube at least one-half the length of the conidium) was assessed microscopically.

Assessment of initial colony establishment on cold pretreated leaves. Conidial germination, appressorium formation, and primary, secondary, and higher-order branching of hyphae were assessed on inoculated leaves as follows: Twenty-four hours after detached leaves were exposed to an acute cold event of 2, 4, 6, or 8°C for 2 or 8 h, conidia were dusted onto the upper leaf surface by tapping the lower surface of an inverted, heavily-infected seedling leaf suspended approximately 2 cm above the leaf to be inoculated. Forty-eight hours postinoculation (hpi) leaves were fixed overnight in a 3:1 glacial acetic acid: 95% ethanol solution, then were stored in a 50% ethanol solution until they could be assessed. To visualize the conidia, leaves were soaked for 30 s in a staining solution of 1000 ml methanol, 800 ml water, 200 ml glacial acetic acid and 60 ml Commassie Blue filtered working solution, containing 0.25 g Commassie Brilliant Blue G (Sigma Chemical Company, St. Louis, MO, USA), 90 ml 50% methanol, and 10 ml glacial acetic acid. After staining, leaves were rinsed in distilled water, soaked in 25% glycerol for 10 min and then mounted on glass slides in 50% glycerol. The first 100 germinated conidia per replication were counted using compound microscopy with 100X magnification and categorized as: (i) germination with appressorium formation, (ii) primary hypha development, or (iii) branched and multiple hyphae. Non-germinated conidia were not counted, since previous research had shown that germination is independent of the nature of the physical characteristics of the substrate (7, 8) and the acute cold treatment occurred before inoculation. Three leaves per treatment were used, and the experiment was conducted four times. Conidial counts were converted to percent-in-class, as the total counts across each class per replication were 100. Analysis of variance (ANOVA) was used with temperature, duration of exposure, and their interaction as nominal factors for each class of conidial development which were treated as continuous dependent variables. Significance of the levels of both temperature and duration within each developmental

class were compared using least squares mean differences with Tukey's HSD (33). In addition, regression analyses treating temperature as a continuous variable and duration as a nominal factor for each conidial developmental class was performed.

Assessment of colony development on cold pretreated leaves. Colony expansion over a longer period of development was also measured. Twenty-four hours after detached leaves were exposed to an acute cold event, which consisted of a 2, 4, 6 or 8°C exposure for 0 (control), 2, 4, 6 or 8 h, they were inoculated using a spore suspension of 10^5 conidia/ml in 0.01% Tween 20 solution. Ten 5 µl drops were dispensed onto the upper surface of each leaf. The drops were allowed to dry at room temperature for 30 min before the leaves were returned to incubation at 22°C. Five days postinoculation (dpi), colony diameter was measured under a dissecting microscope by measuring the length of two axial transects through the center of the colony. Total colony area was calculated using the area of a circle (πr^2) and the average of the two transects for diameter. This was converted to a percentage of the control treatment. Three replicate leaves were inoculated per treatment and the experiment was conducted four times. Effects of temperature and duration treatments on colony area were analyzed using ANOVA, with temperature and duration as nominal independent factors. Tukey's HSD was used to test for significant differences among levels within each factor. Bartlett's test for homogeneity of variance between the temperature factors at a given duration was used to confirm that pooling of temperature at a given duration could be performed (33).

Comparing detached and attached leaves. As an additional control treatment to ascertain possible artifacts induced by detachment of host leaves, we repeated the above experiment using both detached leaves of *V. vinifera* 'Cabernet franc' and leaves attached to potted whole-plant 5-year-old *V. vinifera* 'Cabernet franc' grapevines. Leaves and whole plants were subject to an acute cold event of 2°C for 8

h. Three leaves and three vines (1 leaf per vine) were used per treatment, inoculated and incubated as described above. Control vines were maintained at 22 to 25°C. Colony diameter was measured on each of three leaves inoculated with a spore suspension as described above. The experiment was conducted twice. The coefficient of variation was calculated for host type (whole plant and detached leaf), and used to compare the magnitude in variation of cold treatments on observed effects. In addition, Student's *t*-test was used to compare the magnitude of the cold treatment effect on colony size between whole plant and detached leaves.

Transient effects of preinoculation exposure of grapevine leaves to acute cold temperature events. The most ontogenically susceptible leaves (as described above) of 5-year-old potted *V. vinifera* 'Cabernet franc' vines were collected, surface disinfested and placed on 1% water agar plates as described above. Treatments consisted of a single exposure of 2°C for 8 h, with controls remaining at 25°C. At 12, 24, 36 or 48 h posttreatment, leaves were either dusted with conidia for development assessment at 48 hpi using the Commassie Blue staining technique as described above, or inoculated using a spore suspension to observe effects on colony expansion at 5 dpi as described above. Three replicate leaves were used per experiment, and the 12 and 24 h experiments were repeated twice, while the 36 and 48 h experiments were repeated once. Treatment effects were analyzed using ANOVA, and treatment factors were compared to the controls using Dunnett's method (33).

Effects of acute cold events on *in vitro* germination and appressorium formation. Conidia of *E. necator* from freshly sporulating detached leaf cultures were dusted onto glass microscope slides in Petri dishes containing moist filter paper to maintain a constant, saturated humidity. The slides were then split into two groups: (i) incubated at 25°C for 24 h, and (ii) incubated at 2°C for 6 h and then transferred to 25°C for another 18 h. Thereafter, the slides were examined at 400 X magnification

and at least 100 conidia per slide were rated for germination (i.e., presence of a germ tube at least one-half the length of the conidium) and presence of an appressorium. Each treatment was replicated on three slides and the experiment repeated. Percent germination was calculated from total observed conidia per slide and standard errors were computed. A Student's *t*-test was performed to determine statistical significance of the treatment effect.

Exposure of nascent mildew colonies to acute cold events. The most ontogenically susceptible leaves (as described above) of 5-year-old potted *V. vinifera* 'Pinot noir' vines were inoculated using 5 µl droplets of a freshly-prepared aqueous suspension containing 10^5 conidia/ml and 0.01% Tween 20. Ten droplets were placed on the upper surface of each of three leaves of six plants per treatment. Of the six plants per treatment, three were reserved for the assessment of colony development while the remaining three were destructively sampled to assess colony viability. Colonies were allowed to develop for 24 h in the greenhouse at 22 to 25°C with 16 h supplemental lighting as described earlier, before exposure to a cold event.

Colonies were exposed to an acute cold temperature event by placing the potted vines on the floor of a research vineyard at the New York State Agricultural Experiment Station (NYSAES) at Geneva, NY from 3 p.m. to 8 a.m. beginning on 29 April 2008. The experiment was repeated on 1 May 2008. Ambient temperature was recorded every 15 min by a CR10X datalogger (Campbell Scientific, Inc. Logan, UT, USA) located approximately 1 km from the site. Control potted vines were maintained in a greenhouse at 22 to 25°C with supplemental lighting as described.

Colonies borne on inoculated leaves of the three vines reserved for assessment of colony development were examined under a dissecting microscope at 16 X while still attached to the potted vines 6 days after inoculation (i.e., 5 days after exposure to an acute cold temperature event in the vineyard). Colony development was

categorized as (i) hyphal growth only; (ii) conidiophores developed, but non-sporulating; and (iii) sporulating colony. Student's *t*-test was used to compare the number of colonies from the cold and control treatments that developed to each category. On the three vines reserved for destructive sampling, mortality of hyphae within developing colonies was assessed 4 dpi (i.e., 3 days after exposure to an acute cold temperature event) by immersing detached leaves in a 1:1000 aqueous dilution of 0.5% (w/v) fluorescent vital stain fluorescein diacetate (FDA) (Aldrich Chemical Co., Milwaukee, WI, USA). After 3 min, colonies were examined using fluorescence microscopy (325 to 500 nm excitation filter, and transmission filter above 530 nm) and the percentage of the colony area not exhibiting bright green fluorescence were visually estimated. Mean non-fluorescence between the cold-exposed and control colonies were compared using Student's *t*-test.

The above experiment was repeated under controlled conditions with some modifications. Seedlings were grown from stratified seed harvested from open-pollinated *V. vinifera* 'Riesling'. The most ontogenically susceptible leaves were detached from the seedlings at the petiole, placed onto 1% (w/v) water agar plates, dusted with conidia of *E. necator* as described above, and incubated at 24°C. On 2, 3, 4, 5 or 6 days postinoculation (dpi), selected leaves were moved to a 2°C incubator for 8 h (dark) and returned thereafter to 24°C. Three replicate leaves were used for each time point. Twenty-four hours after the end of the acute cold temperature events, leaves were flooded with FDA and hyphal mortality within 15 colonies was assessed under fluorescence microscopy as described above. The experiment was repeated five times for those leaves assessed at 2, 3, 4, and 5 dpi, and twice for those leaves assessed 6 dpi. Percent colony mortality for both the treated and controls colonies, within each colony age category, was analyzed using Student's *t*-test for mean comparisons as each colony age experiment was conducted at separate times. Colony mortality in the

cold treatment within each age category was converted to percent mortality relative to the control by the following equation:

$$X = (Y-Z)/Y*100 \qquad \text{(Equation 2.1)}$$

where X= percent colony mortality relative to control; Y= percent colony mortality in the cold treatment; and Z= percent colony mortality in the control treatment.

Development of mildew colonies on mature field-grown vines exposed to natural acute cold temperature events. Experiments were conducted in a vineyard of *V. vinifera* 'Chardonnay' planted in 2004 at NYSAES wherein vines were inoculated sequentially throughout the growing seasons of 2008 and 2009. Vines were maintained under a calendar spray program to reduce the possibility of natural infection, and leaves were visually inspected for powdery mildew prior to inoculation. If a scheduled spray occurred within 5 days of inoculation, shoot tips were protected from fungicide residue with a food-grade plastic bag during the fungicide application which allowed emerging tissue to remain free of residual pesticides. The most ontogenically-susceptible leaves (see above) were inoculated every 3 to 7 days beginning on 22 May 2008 and on 19 May 2009, approximately 3 weeks after budbreak in each year, respectively. Ten 5 µl drops of a 10^5 conidia/ml suspension were applied to each of 24 leaves per inoculation date. Germination potential of conidial suspensions was assessed on glass slides as described above, and also by placing 5 µl droplets of the conidial suspension on glass slides that were affixed to shoots of the inoculated vines and left to incubate overnight in the vineyard. Three days postinoculation, 3 leaves were harvested and colony development and hyphal mortality was assessed as above. Sampling occurred every 3 days until colonies began to develop tertiary branching and conidiophore initials.

Predicted latent period under variable field temperatures (collected hourly, as an average of measurements taken at 15 min intervals) was derived by fitting two

linear regression equations to data reported by Delp (7) on the duration of the latent period at various constant growth chamber temperatures:

If T ≤ 10°C then L= 1272-84*T **(Equation 2.2)**

If 10°C<T<31°C then L=28.68+2839*(1/T) **(Equation 2.3)**

where T = average hourly temperature and L = the predicted latent period in hours. The predicted latent period (L) was then transformed to 1/L to represent the total fraction of the period elapsed during an hour at the given temperature. The values of 1/L were summed beginning at the time of inoculation until the sum was ≥1, at which time the predicted latent period was complete. The predicted latent period in hours was then converted to days (rounded to the nearest integer) for comparison to latent periods observed in the above vineyard experiments. Regression analysis was used to compare the difference between observed and predicted latent period values as it relates to the timing of inoculation. When lines appeared to have a classic broken-stick form, the growing season was divided into two groups (i) budbreak to bloom and (ii) postbloom, and comparisons between average predicted and observed field latent periods were done using Student's *t*-test.

Frequency of occurrence of acute cold temperature events in diverse viticultural regions. As part of an international research project to assess the impact of climate on heterogeneity of grapevine phenology (20), hourly temperature data were collected during the 2005, 2006 and 2007 growing seasons in vineyards in the following locations: Geneva, NY, USA; Davis, CA, USA; Raleigh, NC, USA; Hobart, Tasmania, Australia; Loxton, South Australia, Australia; and Bernkastel, Germany. For the present study, data from each site were reanalyzed to determine: (i) the average minimum temperature of the period from budbreak to bloom, calculated from the daily minimum temperatures, and (ii) the mean number of days between budbreak and bloom during which the minimum temperature was below 6°C.

Disparity between ambient air temperatures and leaf surface temperatures under vineyard conditions. To assess the degree to which leaf surface temperatures differed from ambient air temperatures during the night hours during which acute cold temperature events occurred, ambient air temperature 2 cm above the leaf was measured using thermocouples (Omega HH506R thermometer with T-type thermocouples, Omega Engineering, Inc, Stamford, CT, USA; accuracy of 0.05% ±0.3°C). Direct measurements of leaf surface temperature were obtained using a M125E Series Portable Infrared Thermometer (Mikron, Oakland, New Jersey, USA; accuracy of 1% ±1°C) held within 50 cm of the leaf (measuring temperature over a 3 cm diameter circle according to manufacturer's specifications). Temperature measurements were taken at 7:30 p.m. and 5:20 a.m. on 12-13 May 2009, 17-18 May 2009 and 18-19 May 2009. Leaf surface temperature was recorded as the mean of three instantaneous readings taken on each of two leaves. Air temperature 2 cm above the same leaves was recorded as the mean of three instantaneous thermocouple measurements. Cloud coverage was also recorded for each observation date. Differences between leaf surface temperature and ambient air temperature at each time point were compared using Student's *t*-test. Regression analysis of the difference between predicted and observed latent periods versus the minimum low temperature experienced within the first three days of development in the field was done to see if the observed differences were due to in-field cold temperature events during the critical stage of colony development.

Statistical analyses. All statistics beyond mean and standard error calculations were performed using JPM Statistical Software (SAS Institute, Inc., Cary, North Carolina, USA). The "One-Way" analysis was used for ANOVA, and the "Fit Model" platform with "Standard Least Squares" was used for regression analysis.

RESULTS

Preinoculation exposure of grapevine leaves to acute cold temperature events. *Assessment of initial colony establishment on cold pretreated leaves.* Preinoculation exposure of seedling leaf tissue to acute cold temperature events affected the development of conidia applied to those leaves in comparison to the controls. The effects of temperature and treatment duration on colony development were significant for developmental class (i), appressoria only, (P=0.014 and P=0.004, respectively), and developmental class (iii), branched hyphae, (P=0.005 and P=0.001, respectively). There was no interaction effect between temperature and duration (P=0.52 for developmental class (i) and P=0.61 for developmental class (iii)). There were no significant effects of temperature, duration or their interaction on the number of conidia developed to class (ii), primary hyphae, (P=0.12, 0.11, 0.93, respectively). Within effects test of the temperature levels for the developmental classes (i) and (iii) showed that 2 and 8°C were significantly different from each other, but 4 and 6°C were not significantly different from either 2 or 8°C (P>0.05). In all cases, the percentage of conidia with appressoria decreased with increasing temperature while the percentage of conidia with branched hyphae increased with increasing temperature. Within effects test for treatment duration for both developmental classes (i) and (iii) indicated that 2 and 8 h treatments were not significantly different from each other (P>0.05), but were significantly different from the controls (P<0.05). To limit redundancy of presentation, and because the 2 h treatment was more representative of the duration of acute cold temperature events under field conditions in the early growing season, only the data for the 2 h treatment are shown in Fig. 2.1. Regression analysis, where temperature was treated as continuous rather than nominal, showed that the percentage of germinated conidia that failed to progress beyond the formation of appressoria within 48 hpi was highly correlated with and inversely

proportional to the temperature of the preinoculation cold temperature event (Fig. 2.1A, R^2=0.99, P=0.006 for the slope coefficient). A similar relationship was found when the temperature of the preinoculation cold temperature event was increased from 2 to 8°C (Fig. 2.1B), where a progressively higher percentage of conidia advanced to form secondary hyphae (R^2=0.92, P=0.04 for the slope coefficient). Exposure to the control temperature (24ºC) resulted in conidial development that was not significantly different from the 8ºC exposure for all three developmental classes.

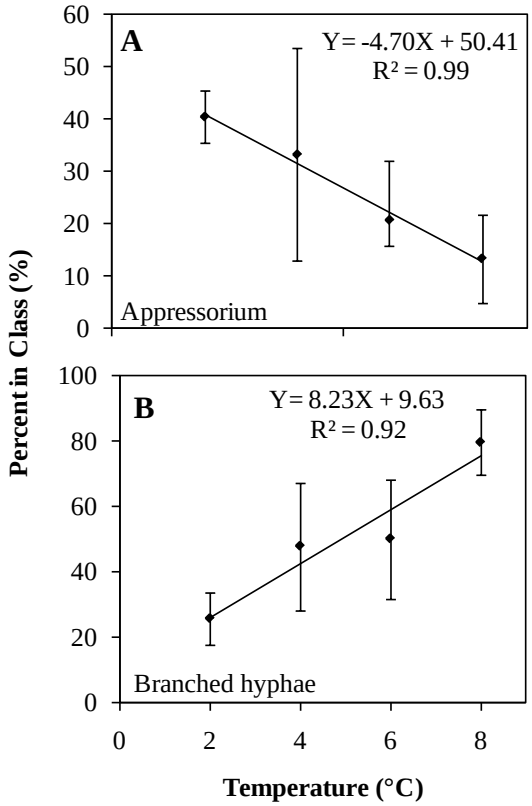

Figure 2.1- Effect of cold pretreatment of *Vitis vinifera* 'Chardonnay' leaves on initial infection by conidia of *Erysiphe necator*. Detached leaves were exposed to temperatures ranging from 2 to 8°C for 2 h prior to inoculation. Fungal development was assessed 48 hpi for the percentage of germinated conidia that had progressed no farther than the formation of: A) appressorium, and B) branched hyphae.

Assessment of colony development on cold pretreated leaves. In a separate experiment to see the longer-term effects of exposure of seedling leaves to acute cold temperature events prior to inoculation, cold temperature exposure resulted in a reduction in the relative area of 5-day-old colonies of 37 to 55% compared to unexposed colonies (Fig. 2.2). In contrast to the effects on initial colony establishment as described above, ANOVA analysis showed that the degree of suppression of colony area relative to the control was not related to the extent (level of temperature) of the acute cold event (effects test P=0.41 for temperature treatment). However, the simple occurrence of a cold event, i.e., duration greater than 0 h (control) at a given temperature treatment, did significantly affect colony size. The durations of the temperature treatments from 2 to 8 h were not significantly different from each other according to Tukey's HSD (P>0.05).

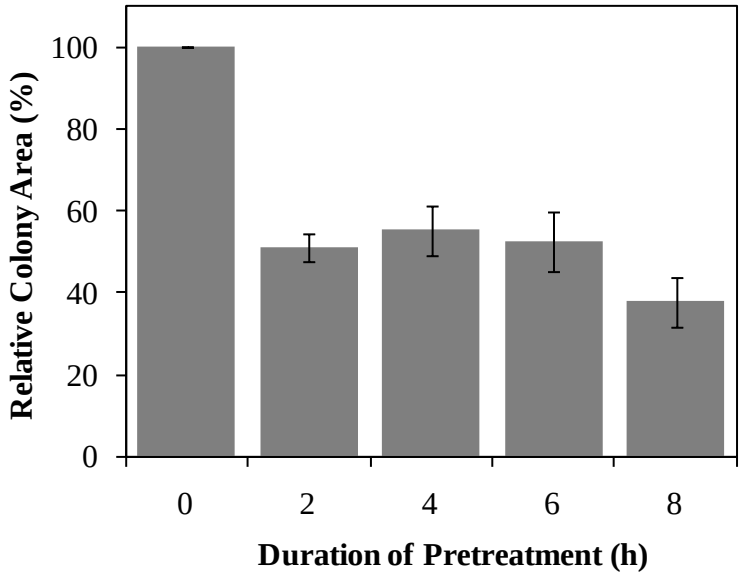

Figure 2.2- Effect of pre-inoculation cold treatment of *Vitis vinifera* 'Chardonnay' leaves on subsequent *Erysiphe necator* colony development at 5 dpi. Colony area was expressed as percent of the control treatment (0 h), and data were pooled across all temperature treatments from 2 to 8°C.

Comparing detached and attached leaves. The magnitude and variation of the treatment effect was similar between detached leaves and attached leaves on potted *V. vinifera* 'Cabernet franc' when both were exposed to 2°C for 8 h; i.e., colonies that developed on cold-treated detached leaves expanded to 81% the size of those grown on control leaves with a coefficient of variation (CV) of 0.38, compared to 77% with a CV of 0.42 for colonies on cold-treated attached leaves. The treatment effect on colony size was not statistically different between the detached and attached leaves (P=0.43).

Transient effects of preinoculation exposure of grapevine leaves to acute cold temperature events. Cold induced resistance is transient when compared to controls (P<0.0001, Dunnett's method), with a maximum treatment effect at 24 h after the occurrence of a cold treatment. In the assessments at 48 hpi, significantly more conidia failed to infect in the 24 h treatment, 26% of total conidia compared to 11% in the control treatment (P=0.007). In the other treatments, with inoculations at 12, 36 and 48 h, 14, 14, and 13% of total conidia failed to infect, and were not significantly different from the control (P=0.94, 0.94 and 0.99, respectively). The 24 h treatment also had significantly fewer nascent colonies with branched hyphae, 52% of the total, compared to the 81% in the control treatment (P=0.015). The other treatments, with 12, 36 and 48 h inoculations, had 69, 67 and 72% of total nascent colonies with developed branched hyphae, and were not significantly different than the control treatment (P=0.56, 0.57 and 0.85, respectively). Similar results were found when colonies were observed at 5 dpi. The 24 and 36 h inoculation treatments resulted in mean colony areas that were significantly smaller than the controls (P<0.0001 and P=0.0014, respectively), whereas the 12 and 48 h inoculation treatments resulted in mean colony areas that were not significantly different from the controls (P=0.53 and 0.37, respectively).

Effects of acute cold temperature events on in vitro germination and appressorium formation. Exposure of conidia on glass slides to a 6 h cold event at 2°C had no effect upon subsequent germination or appressoria development. Conidial germination was reduced by 1.24% (SE=3.94%) and wasn't significantly different from the control (P=0.75), and appressoria formation was reduced by 2.39% (SE=5.00%) and wasn't significantly different from the control (P=0.65), when exposed to a 6 h cold event.

Exposure of nascent mildew colonies to acute cold temperature events. In field experiments, the outdoor cold event occurring on 29-30 Apr, resulted in significantly fewer colonies in development category (iii), sporulation, (P=0.001), and significantly more colonies in development category (i), hypal growth (P=0.01), at 5 dpi posttreatment compared to controls. The temperature range over the course of the 29-30 Apr outdoor treatment was 0.0-8.5°C. The outdoor cold event occurring on 1-2 May did not result in a significant difference between treatment and control colonies either development categories (i) or (iii) (P=0.23 and 0.59, respectively). The temperature range over the course of the 1-2 May outdoor treatment was 8.4-14.0°C. In both field and controlled environment studies, mildew colonies exposed to acute cold temperature events had a significantly greater proportion of the colony that did not hydrolyze FDA (presumed non-viable) than colonies without cold temperature exposure (P<0.0001, e.g. Fig. 2.3). In the field, colonies exposed to the overnight cold event of 29-30 Apr 2008 (minimum ambient temperature =0.0°C) exhibited a mean rate of non-fluorescence of 46.8% (SE=4.1%) compared to 14.7% (SE=2.7%) in non-exposed controls, and the cold treatment had a significantly higher level of non-fluorescence (P<0.0001). Those exposed to the overnight cold event of 1-2 May 2008 (minimum ambient temperature 8.4°C) exhibited a mean rate of 61.4% (SE=4.9%)

compared to 28.8% (SE=3.5%) in non-exposed controls, and the cold treatment had a significantly higher level of non-fluorescence (P<0.0001).

In controlled environment studies, the effects of an acute cold temperature (2ºC) event of 8 h on nascent mildew colonies ranging in age from 2 to 6 days-old, were only significant on 3-day-old colonies. While the other age categories trended towards reduced fluorescence in the cold treatment, they were not statistically different from their respective controls (Table 2.1).

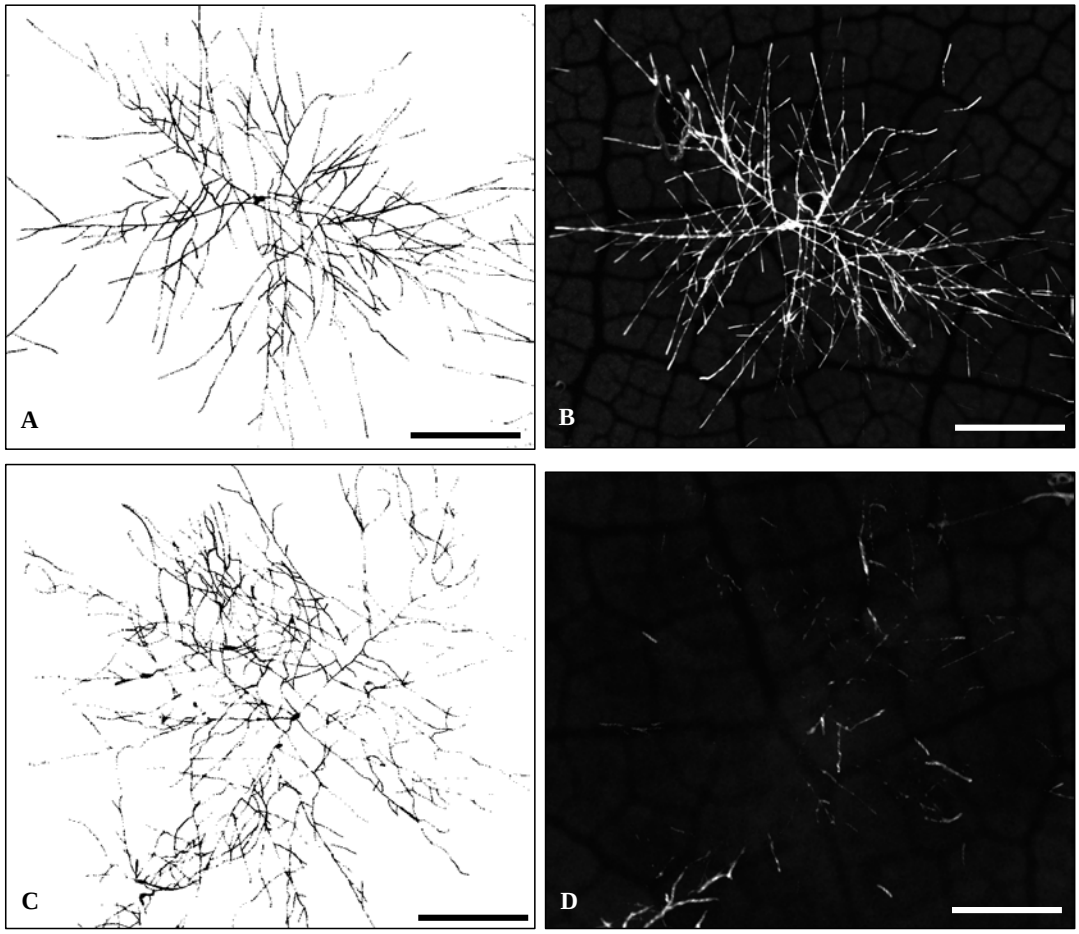

Figure 2.3- Line sketches and vital staining of 4-day-old *Erysiphe necator* colonies growing on detached *Vitis vinifera* 'Chardonnay' leaves under controlled conditions. A) Line sketch of a colony grown at a constant optimum temperature of 24°C, B) The same colony stained with FDA and exposed to UV light, C) Line sketch of a colony grown at 24ºC save for acute exposure to 2°C for 8 h at 3 dpi, and D) The same cold-treated colony stained with FDA and exposed to UV light. Scale bars are 50 µm.

Table 2.1- Percent mortality (non-fluorescence) in young *Erysiphe necator* colonies on *Vitis vinifera* 'Riesling', exposed to an acute cold temperate (2°C for 8 h).

Timing of cold exposure[w]	Total colony mortality-Control (%)[x]	Total colony mortality-Cold-treated (%)[x]	Mortality relative to controls (%)[x,y]	(Student's *t* value)[z]
2 dpi	50.5 (8.5)	57.1 (18.7)	11.6	*P*=0.71
3 dpi	20.6 (7.7)	61.5 (4.3)	66.7	*P*=0.005
4 dpi	20.3 (10.1)	47.4 (3.9)	57.2	*P*=0.07
5 dpi	22.5 (9.2)	44.9 (8.8)	49.9	*P*=0.13
6 dpi	43.7 (2.2)	64.4 (13.0)	32.1	*P*=0.16

[w] Timing measured as days post inoculation (dpi).
[x] Standard error in parenthesis.
[y] Calculated as percent increase [(Cold Treated- Control)/Cold Treated*100].
[z] Comparing mean colony mortality for cold treated and control colonies at each development stage.

Development of mildew colonies on mature field-grown vines exposed to natural acute cold temperature events. When the differences between actual and predicted latent periods was regressed against climate day (where calendar day numbering starts on 1 Mar), the slope was significantly different from zero (*P*=0.02). Observations were then divided into two groups, (i) budbreak to bloom, and (ii) post bloom. Latent periods, following inoculations performed during the period between budbreak and bloom (climate day 62 to 107), were 75% longer than predicted by Eqs. 2.2 and 2.3 (*P*=0.0006), whereas latent periods following inoculations between bloom and veraison were 30% longer than predicted (*P*=0.04) (Fig. 2.4). The magnitude of the disparity between observed and predicted latent period was significantly correlated (*P*=0.0001) with the minimum temperature that occurred within 3 dpi with (Fig. 2.5). Colonies that were 3 days old on 31 May 2009, when overnight temperatures dropped below 5°C for 8 h, had not sporulated when observations were terminated 24 d later,

although the predicted latent period for this cohort of colonies was only 8 days. Similarly, colonies resulting from inoculations made 2 d after this same cold event did not sporulate until 20 dpi, or 13 d after the end of the latent period predicted for this cohort of colonies.

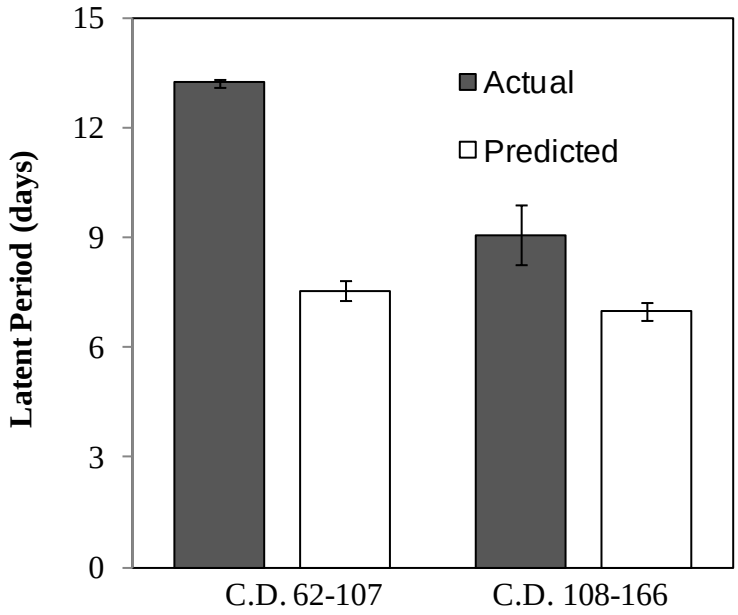

Figure 2.4- Observed and predicted latent periods of *Erysiphe necator* on field-grown *Vitis vinifera* 'Chardonnay' in 2008 and 2009 relative to climate cay (day 1=1 Mar). Observed latent period is the time from inoculation to first sporulation, predicted latent period was derived from Equations 2.2 and 2.3 using hourly weather data.

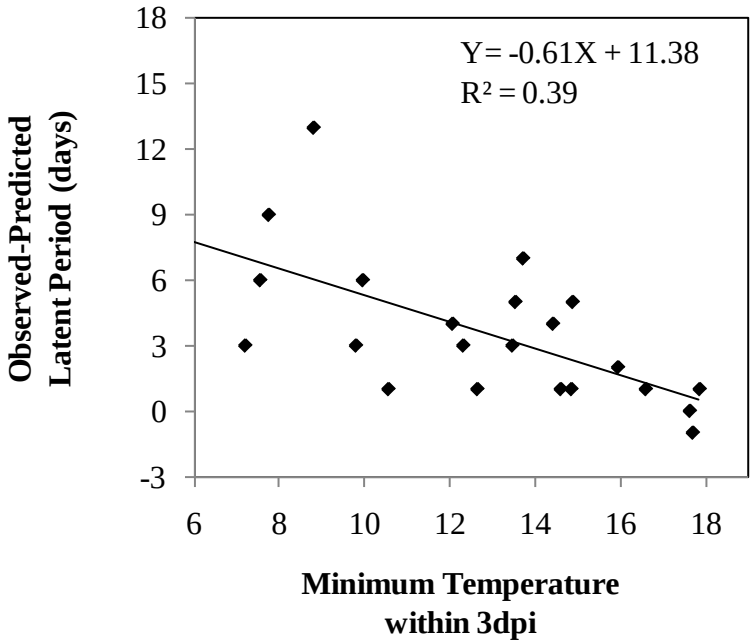

Figure 2.5- Difference in observed versus predicted latent period for *Erysiphe necator* on *Vitis vinifera* 'Chardonnay' as it relates to the minimum temperature experienced in the field within 3 dpi. Observed latent period was the time between inoculation and first sporulation; predicted latent period was derived from hourly temperature measurements using Equations 2.2 and 2.3 in the text.

Frequency of occurrence of early-season cold temperature events in diverse viticultural regions. Across a broad range of climates in the northern and southern hemispheres, overnight low temperatures in vineyards fell below 6°C an average of 14 to 21 times between budbreak and bloom (Table 2.2). The average overnight minimum temperature for the period delimited by the above phenological stages ranged from 6.0°C at Hobart, Tasmania to 10.2°C at Raleigh, North Carolina (Table 2.2). Although the average low overnight temperature was higher at Loxton, South Australia (8.9°C) than at Geneva, NY (7.1°C), both sites experienced an average of 17 nights when temperatures fell below 6°C (Table 2.2).

Table 2.2- Summary of overnight cold temperature events between budbreak and bloom occurring in six viticulture regions around the world.

Site	Climate Type	Average minimum temperature (°C)[x]	Days between budbreak and bloom with minimum temperatures ≤ 6°C[y]
Geneva, New York	Cool temperate	7.1	17
Hobart, Tasmania	Maritime	6.0	21
Davis, California	Mediterranean	7.6	16
Bernkastle, Germany	Northern temperate	7.5	18
Loxton, South Australia	Mediterranean/desert	8.9	17
Raleigh, North Carolina	Warm temperate	10.2	14

[x] Average daily minimum temperature of the period between budbreak and bloom.
[y] Integer average of period from budbreak to bloom for 2005 to 2007 inclusive.

Disparity between ambient air temperatures and leaf surface temperatures under vineyard conditions. In all overnight measurements, the temperature of the leaf surface was substantially and significantly (P=0.05 to <0.0001) below that of the air 2 cm above the leaf (Table 2.3). The differential between the leaf surface and air temperature ranged from -0.9°C to -7.6°C (Table 2.3). The differences between leaf surface and air temperatures (-3.8 to -7.6°C) were greatest at 7:30 p.m. compared to 5:20 a.m. on all three days (Table 2.3). This was approximately 1 h before sunset on the dates of observation, although differences of -3.0 and -3.4°C were observed at 5:20 a.m., approximately 30 min before sunrise on the dates of observation (Table 2.3). Temperature differentials of approximately the same magnitude were recorded under both clear and overcast conditions, and under calm conditions, and in the presence of light wind (Table 2.3).

Table 2.3- Leaf surface and air temperature in a vineyard of *Vitis vinifera* 'Chardonnay' approximately 2 weeks after budbreak at Geneva, NY.

Date	Time[x]	Leaf[y] (°C)	Air[y] (°C)	Δ T[z] (°C)	Cloud Cover	Wind Speed (km/h)
12-13 May	7:30 p.m. 5:20 a.m.	13.0 (1.4) 0.5 (0.2)	16.8 (0.6) 3.5 (0.1)	-3.8 (P<0.0001) -3.0 (P<0.0001)	Clear Clear	0 0
17-18 May	7:30 p.m. 5:20 a.m.	5.7 (1.9) 3.7 (0.5)	11.3 (0.0) 4.6 (0.0)	-7.6 (P=0.002) -0.9 (P=0.05)	Clear Overcast	0 <10
18-19 May	7:30 p.m. 5:20 a.m.	4.8 (2.1) -1.7 (0.5)	11.4 (0.4) 1.7 (0.1)	-6.6 (P=0.002) -3.4 (P<0.0001)	Clear Clear	0 0

[x] Times of measurements were approx. 1 h before sunset and 30 min before sunrise.
[y] Temperature as indicated by infrared sensing of leaf surface, or a thermocouple located 2 cm above the leaf surface. Values in parenthesis are standard errors.
[z] Difference between leaf surface temperature and air temperature 2 cm above leaf surface. Values in parentheses indicate significance by Student's *t*-test.

DISCUSSION

Leaves that were normally ontogenically susceptible yielded a more resistant phenotype when exposed to an acute cold temperature event. Exposure of grapevines to acute cold temperature events affected several fitness parameters and was deleterious to the development of powdery mildew epidemics at multiple levels. Infection efficiency (ability of a conidium to progress beyond appressorium within 48 h of arrival on the host) was reduced, resulting in the development of fewer colonies from a given number of germinated conidia. Radial expansion of colonies was slowed (i.e., smaller colonies at 5 dpi on cold pretreated leaves). Within established colonies, individual hyphal segments died thereby reducing colony density. Finally, the field latent period was lengthened, in some cases more than doubled, in association with acute cold temperature events occurring early in the growing season. The foregoing pathogen responses were similar to those reported to occur during the transition of

ontogenically susceptible grape berries to a resistant state as they age (11, 12, 15, 16, 19, 23), e.g., conidia placed upon ordinarily susceptible leaves previously exposed to acute cold temperature events produced a phenotypic response that would commonly be observed on ontogenically resistant berries.

Exposure of grape leaves to an acute cold temperature event before inoculation reduced the percentage of germinated conidia that were able to form hyphae beyond a germ tube. This is a prepenetration-like resistance phenotype seen in grape berries (13, 23) and appears to be the consequence of an occurrence, but not the duration, of cold pretreatment. We also observed that this cold-induced resistance was transitory with the greatest resistance response occurring between 24 and 36 h after a low, non-freezing temperature event. This transitory response may be associated with a number of equally ephemeral responses of plants to acute cold (43).

In contrast to the effects of cold treatment on initial colony establishment, effects upon colony expansion were still apparent at 5 dpi (6 days after cold pretreatment). The occurrence of a cold temperature event alone was sufficient to elicit a significant treatment effect independent of the degree or duration of such an event (Fig. 2.2). Unlike the foregoing studies on conidial germination, colonies were established by a method that deposited a concentrated dose of inoculum (a 5 µl droplet containing 500 conidia), thereby increasing the probability of establishment at any given point. The different results with respect to duration and intensity of cold temperature events among germlings compared to colonies are not necessarily contradictory. In the studies on germinated conidia, spores were dusted onto the tissue and were widely separated. Thus, in the case of the germinated spores, we recorded the effects of acute cold temperature events upon the success or failure of individual conidia development, whereas establishment of a colony was a near certainty due to

the quantity of inoculum deposited using the conidial suspensions. Thus the responses are not directly comparable.

Nascent colonies directly exposed to acute low temperature events exhibited higher mortality among hyphal strands within colonies (Table 2.1 and Fig. 2.3). This result is similar to that reported for damage rendered by exposure to high temperatures and ultraviolet radiation (4, 7, 49), as well as development of ontogenic resistance (11, 12, 15, 16, 19, 23). The lack of ability in the 2 and 6 dpi controls to hydrolyze FDA was likely an artifact of the FDA staining process on very young or aging colonies: slow growth and metabolism can result in reduced fluorescence (28, 41). While the same could be said for the cold treated colonies, reduced fluorescence as a result of cold exposure when compared to controls indicated that this acute cold stress was reducing the metabolic activity of the nascent colony. In the present study, a statistically significant reduction in fluorescence was observed when 3-day-old colonies were exposed to acute cold temperature events. This is a particularly critical stage in the development of a powdery mildew colony, as under the optimal temperature conditions used before and after acute cold temperature exposure, the 3-day-old colonies would be in transition from vegetative growth to reproductive development (7). Furthermore, in vineyards the magnitude of the disparity between expected and observed latent period was correlated with the occurrence of acute cold temperature events within 3 days after inoculation (Fig. 2.5). While previously described temperature relationships treated low, non-freezing temperatures (temperatures less than 10ºC) as non-killing (7, 36) our data showed that acute low temperature events have a clear negative impact upon the pathogen.

Low, non-freezing temperatures are known to retard developmental processes in plants, alter source/sink relationships, reduce carbon assimilation (26), alter stomatal behavior (49), increase ethylene production resulting in dwarfing (27), and

reduce vigor (6). Substantial work done in *Arabidopsis* on the response to cold temperatures has shown that cold stress response is often similar to other stress and water relation responses (Thomashow (43) provides an excellent review), and recent work has implicated the involvement of the salicylic acid (SA) pathway in response to chilling (46). In grapes, exogenous application of SA appears to induce cold tolerance (45). Near-freezing temperatures also are associated with increased resistance to a variety of pathogens of grasses, including snow molds (1, 22), *Bipolaris sorokiniana* on barley (35), and *Puccinia poae-nemoralis* on bluegrass (44). With respect to *E. necator* on grapevine, reduced vigor and host stress is generally associated with attenuated growth of the biotrophic pathogen, and reduced disease severity(3, 34). Thus, the impact of acute low temperature events may be exerted through either or both of the following: (i) a deleterious effect of acute low temperatures on host growth and development that makes tissues of lower quality as a substrate for pathogen growth, or (ii) triggering or enhancement of biochemical pathways associated with stress and disease resistance, particularly in rapidly developing young tissues. What distinguishes our study from most of the foregoing is that the responses we noted appear to be transient and to some degree reversible. The increased resistance of young leaves reported here for *V. vinifera* seedlings is not due to repeated or prolonged exposure to gradually decreasing temperatures associated with winter dormancy. The phenomena we observed in our field studies were associated with punctuated, random events during what typically spans the postdormancy period for temperate woody plants. This is when they are generally considered to be losing their frost/freezing resistance (25) and are the most susceptible to frost damage. While the genetics of cold acclimation and chilling/freezing stress in grapevine have been investigated (21, 42), we are not aware of previous reports indicating that chilling stress functions as an inducer of resistance to powdery mildew.

A substantial increase of host resistance due to acute cold temperature events was demonstrated in our preinoculation studies. Our postinoculation studies were not designed to separate the direct effects of treatments on the pathogen from those exerted through the host. It will be challenging to definitively separate direct and possibly deleterious effects of acute cold temperatures on an established biotrophic pathogen from effects caused by cold-induced changes in host susceptibility. However, the results of our preinoculation experiments, the lack of an effect of acute cold temperature events on *in vitro* germination and appressorium formation, together with previous studies cumulatively suggest that a resistance response in the host could be responsible for a major proportion of the observed effect on nascent colonies. In the vegetative state, *E. necator* survives freezing temperatures within overwintering grapevine buds (34). *Erysiphe necator* colonies on grape seedlings *in vitro* can survive for months at 4ºC and can produce conidia and ascocarps (14). Admittedly, prolonged cold temperatures may have a fundamentally different effect on the pathogen (and host) compared to the transitory cold events with intervening warm periods that typify acute cold temperature events under vineyard conditions. However, we propose that the primary effects of acute cold temperature events are exerted through enhanced resistance of tissues that are normally highly susceptible to infection.

Temperature is the environmental parameter most universally applied to forecast the development of grapevine powdery mildew (5, 24, 36). However, our findings suggest that low, non-freezing temperatures should be taken into greater account than current literature suggests. Some models adjust for low, non-freezing temperatures but either do not treat them as a negative influence on powdery mildew development (36), or classify temperatures between 0 and 15°C as having similar impacts on development (5). Other models assume temperature responses as

instantaneous and without future effect (37), while our data suggests that suboptimal temperature events can affect future development of epidemics. The Gubler-Thomas model (24) does take into effect cooler daytime temperatures and their impact on risk assessment by requiring the satisfaction of 6 or more continuous hours between 21 and 30°C in order to initiate the risk index (with an additional requirement for these conditions to be met for 3 consecutive days) or to maintain risk index values. However, it does not account for punctuated acute cold events that can occur during a 24 h period when daytime temperatures may fall into the optimal development range and thus satisfy the model requirements. The aforementioned acute cold events would be treated as neutral by the Gubler-Thomas model. Our data suggest that cold events should not be considered neutral, but should be taken to have a similar effect on powdery mildew development as acute high temperatures (4, 7, 24). Acute cold temperature events cause a previously unaccounted-for effect that could substantially alter how forecasting systems treat the early-season development of an epidemic.

Our review of climate data demonstrates that acute low temperature events are neither rare, nor are they restricted to otherwise cool climates; they in fact occur with substantial regularity in what are generally regarded as some of the warmest viticultural regions (Table 2.2). Additionally, we found that acute cold temperature events are probably more severe in the field than indicated by ambient air temperatures, as ambient night air was substantially warmer than leaf surfaces due to radiational cooling of leaves (Table 2.3). In previous studies of epidemic progress in grape powdery mildew, several phenomena have been noted that might be related to the occurrence of acute cold temperature events. Gadoury et al., (17) noted that prebloom development of foliar mildew was typified by increased incidence, without a commensurate increase in severity measured as colony size or infected leaf area, i.e., colonies failed to expand despite favorable temperature conditions that existed in the

vineyard. In a study of incidence/severity relationships, Seem (39) reported that foliar severity of grapevine powdery mildew remained both low and nearly constant (i.e., near 1%) regardless of initial incidence levels until foliar incidence exceeded a critical level between 10 and 20%, which occurred after bloom in each year of the study. Collectively, this suggests that acute cold temperature events may suppress the early season spread of grapevine powdery mildew and that present forecasting systems could benefit from modeling this effect.

Our study highlights the impact that a single abiotic stress can have on the development of a biotrophic pathogen. Abiotic stresses such as drought, salt and heat have been reported to reduce host susceptibility in other powdery mildew pathosystems (38, 47, 48). Although we have experimentally examined the impact of single acute cold temperature events, in practice, these events occur repeatedly and their effect could be additive over a period of days or weeks. Furthermore, we have experimentally dealt with ontogenically susceptible tissues, but under field conditions all above-ground tissues of the host could be affected. Thus, the effects of acute cold temperature events might also act in an additive fashion with the already substantial resistance of older foliage and fruit. In continued research, we will investigate both the mechanisms involved in acute cold temperature induced resistance to *E. necator*, and the possible impact of acute cold temperature events on other grapevine pathogens.

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CHAPTER THREE

DESCRIBING GRAPEVINE POWDERY MILDEW EPIDEMICS IN NEW YORK: THE RELATIONSHIP BETWEEN CLUSTER DISEASE SEVERITY AND WEATHER

ABSTRACT

The severity of powdery mildew on grape berries varies substantially from year to year in New York State. Models driven by temperature alone have not accounted for this annual variation in severity of fruit infection. Pan evaporation (E_{pan}), an environmental variable that incorporates temperature, vapor pressure deficit, solar radiation, and wind speed was a better predictor of severity of fruit infection in New York State than temperature alone. Pan evaporation was estimated from evapotranspiration, and forecasted using Monte Carlo simulation for input parameters such as solar radation, wind speed and vapor pressure deficit, coupled with forecasted temperature information. Recursive partition analysis (RPA) provided a simplified decision tree for calculation of powdery mildew risk for New York State vineyards, and incorporated (i) an estimate of the potential primary inoculum levels, and (ii) the current season favorability for pathogen development. This model was verified by comparison with the component data set (observed fruit infection severity for the period of 1985 to 2007) and further validated by comparison to independent fruit infection data from 1975-1984 and 2008-2009. While the use of logistic regression to predict disease severity had fewer instances of misclassification, RPA had few misclassifications and provided a quick means for yearly risk classification. These models, predominately based on in-season E_{pan}, are able to describe potential disease severity in real time or forecast potential disease severity, thereby allowing growers to adapt management programs to present estimates or future forecasts of the risk of berry infection.

Additional Key Words: evapotranspiration, Penman-Monteith, *Erysiphe necator*

INTRODUCTION

The European grape species, *Vitis vinifera*, is widely grown, and includes commercially important cultivars such as ‘Chardonnay’ and ‘Cabernet Sauvignon’. The species is also highly susceptible to powdery mildew, caused by the obligately biotrophic pathogen *Erysiphe necator* (syn. *Uncinula necator*). In relatively cool climates, exemplified by New York, USA or Rheinhessen, Germany, severity of powdery mildew on berries can vary substantially from year to year (20, 28, 32). Additionally, the disease can develop as two distinct but related epidemics: one on foliage and one on fruit. Vines have indeterminate growth that provides successive cohorts of susceptible leaf tissue which acquire incomplete ontogenic resistance. Grape berries are relatively uniform in phenological development. Therefore, they develop ontogenic resistance in a relatively synchronous manner compared to the canopy, resulting in a limited temporal window for fruit infection (17-19, 21).

Successful forecasting of powdery mildew severity on fruit, which is the epidemic of greater economic concern in commercial management of the disease, might require an approach that focuses primarily on disease development on fruit. Several grapevine powdery mildew research and advisory models have been described (7-9, 26, 29, 30), but without a major separation between foliar and fruit epidemics. Some models (9, 35) include rainfall as a limiting factor to epidemic development, or use rain events to serve as an indicator of ascospore (primary inoculum) release. Some models require inputs from repeated scouting for visual detection of disease to initiate management recommendations (26, 36), or the detection of conidia above a specified threshold in volumetric traps (8). All of the aforementioned models generally describe the relative change in the severity of powdery mildew based on past weather, either at the vineyard level (8, 26, 31) or on a single vine (7). They also do so non-specifically with respect to foliar or fruit disease

severity. The models are driven by temperature, particularly the cardinal and lethal temperatures reported for *E. necator* (7, 10, 14, 35). None are designed for disease prediction (*i.e.*, driven by predicted temperature or other environmental parameters).

Several existing models that are primarily temperature-driven (7, 26) have generally over-predicted disease in cooler climates where temperatures lethal to powdery mildew colonies occur rarely in midsummer (8, 28). Powdery mildew colonies are often not detected on foliage in New York State until approximately one month after budbreak (20, 23), and by this time (i.e. approximately 30 May) daily high temperatures consistently fall within with the optimal temperature range of 20-30°C for powdery mildew (14, 32). This characteristic has limited the use of existing models in relatively cool, humid climates. The occurrence of years typified by relatively high or low severity of powdery mildew on fruit of unsprayed, highly susceptible cultivars suggests that macro-scale weather patterns may be involved in annual variations of disease severity on fruit. The synchronous bloom of grapevines that occurs in viticultural regions with relatively cold winters, its relationship to the onset of ontogenic resistance (22), and the resultant narrow timeframe of berry susceptibility (18, 19, 21, 25) further delimits the period during which the effect of such macroscale weather patterns could be exerted. If this is conceptually correct, then the study of weather parameters during a relevant period of crop development could provide a means to forecast favorability for powdery mildew infection of fruit for individual years, across a broad geographic region. The objectives of this study were to (i) define the macro-scale environmental conditions that are historically correlated with both regionally mild and severe powdery mildew infection on fruit clusters in New York State, and (ii) develop an implementable model to help describe this relationship and to aid commercial growers in disease management decisions.

MATERIALS AND METHODS

Historical data. Fruit disease severity data for the years 1974-2009 (expressed as percent of the cluster surface area colonized by *E. necator*) on unsprayed vines primarily of the *Vitis* interspecific hybrid 'Rosette' in research vineyards in or near Geneva, NY were obtained from reports published in Plant Disease Management Reports (formerly Fungicide and Nematicide Tests, American Phytopathological Society). All of these cultivars are considered to be highly susceptible to powdery mildew (2). A summary of collected disease severity data, source, and relevant grapevine phenology is presented in Table 3.1.

Daily weather records for these years were obtained from NOAA benchmark weather station number 3031840 at the New York State Agricultural Experiment Station in Geneva, NY. The station is located at 42°52.6´ N, 77°01.9´ W; is 218 m above sea level; and includes a Class A Meterological Evaporation Pan (E_{pan}). Data from the period 1985 to 2007 were used for initial model development. Weather variables recorded and analyzedincluded minimum, maximum and average daily temperature; daily net solar radiation; average daily vapor pressure deficit; minimum, maximum and average daily relative humidity; total daily pan evaporation; and total daily precipitation. Available hourly data sets included temperature, relative humidity, leaf wetness and precipitation measurements. Dates were logged as Climate Day of Year (CDOY), wherein day 1 is 1 March.

Table 3.1- *Erysiphe necator* cluster severity ratings and related grapevine phenology for the *Vitis* interspecific hybrid 'Rosette' or *labrusca* hybrids in Geneva, NY.

Year	Cluster Severity[x,y]	Budbreak[z]	Bloom[z]	Cultivar
1978	8.0*	62*	113*	Concord
1979	20.0*	62*	113*	Delaware
1980	8.0*	62*	113*	Delaware
1981	10.0	62*	113*	Delaware
1982	n/a	62*	113*	n/a
1983	4.0	62*	113*	Delaware
1984	32.3	62*	113*	Rosette
1985	14.0	64	113	Rosette
1986	48.2	59	103	Rosette
1987	8.2	65	102	Rosette
1988	0.2	70	109	Rosette
1989	47.7	70	111	Rosette
1990	6.4	59	108	Rosette
1991	2.9	62	97	Rosette
1992	50.1	72	118	Rosette
1993	3.4	62*	113	Rosette
1994	36.8	62	109	Rosette
1995	7.3	62*	113	Rosette
1996	31.0	62*	114	Rosette
1997	25.8	62*	117	Rosette
1998	9.4	62*	102	Rosette
1999	4.0	62*	107	Rosette
2000	6.2	62*	115	Rosette
2001	2.5	62*	115	Rosette
2002	4.0	62*	117	Rosette
2003	46.7	62*	120	Rosette
2004	9.0	62*	113	Rosette
2005	11.4	62*	108	Rosette
2006	24.6	68	108	Rosette
2007	8.9	71	105	Rosette
2008	2.8	59	105	Rosette
2009	40.0	60	111	Rosette

[x] Cluster severity as % surface area of cluster diseased. Ratings denoted with an '*' were converted from a 0-3 scale (0=healthy, 3=severely diseased).

[y] Disease data obtained from powdery mildew fungicide trials in *Plant Disease Management Reports*, published by the American Phytopathological Society; formerly *Fungicide and Nematicide* Tests.

[z] Listed as climate day of year (start 1 March). If phenological data were not available, assumption was set as: budbreak 1 May (62) and bloom 21 June (113).

Evaluation of two previously published powdery mildew models in New York. The University California-Davis Powdery Mildew Index (26) and the German-based OiDiag 2.2 expert system (31) were evaluated using weather inputs from the above weather station in Geneva, NY. Modeling outputs and predictions for initial satisfaction of risk thresholds and the timing of subsequent spray recommendations were evaluated with respect to grapevine phenology, and final observed cluster disease severity for each modeled year (Table 3.1).

For the UC-Davis Powdery Mildew Index, the risk accumulation was started on either (i) the first recorded day of observed powdery mildew in the field (Gadoury, *personal communication*), or (ii) 10 days after the first UC-Davis model-defined ascospore release event following budbreak (2.5 mm rain followed by 13 h of leaf wetness and temperatures between 10.0 and 26.0°C). Risk index and accumulation was calculated as described in the model protocol. The years of 1985 through 1991 were used for evaluation as these were representative of a broad range of fruit disease severity ratings, i.e., 0.0% to 48.2% of the cluster surface area infected.

The OiDiag 2.2 expert system was evaluated with the addition of winter temperatures from 1983 and 1984 to calculate the absolute minimum overwintering temperature required in the model protocol (31). The OiDiag 2.2 system calculated the recommended date of the first spray as days after the growth stage corresponding to three unfolded leaves on new shoots. This growth stage was set to 14 d after budbreak based on historical observations of vine growth in Geneva, NY. An additional model input was prior year disease severity (31) estimated from cluster disease severity for Geneva, NY (Table 3.1) and converted to a 1 to 5 scale (Table 3.2) as described in the model protocol (31). The OiDiag 2.2 expert system then calculates the timing of subsequent sprays using a Climatic Index calculated from including daily average temperature, hours of relative humidity between 65 and 80%, hours of relative

humidity >80%, hours of leaf wetness, and total precipitation. This is then multiplied by an Ontogenic Index.

The final output is the OiDiag 2.2 Index Value, which can then be compared to a chart of fungicide products to determine the timing between each spray. The system calculates each day's index value as a running average (31). For the purpose of model evaluation, wettable sulfur was the default fungicide product.

Table 3.2- OiDiag 2.2 expert system disease severity rating scale conversions.

OiDiag 2.2 Severity Rating Scale	Disease Severity on Clusters (Surface Area Infected) (%)
0	0
1	>0-2
2	3-5
3	6-15
4	15-25
5	25+

Development of a New York model. Combinations of linear and logistic regression analyses were used to determine the environmental parameters that yielded the highest correlation coefficients and lowest standard error, and hence greatest predictive value for powdery mildew severity on clusters (expressed as a continuous variable [log transformed]). Weather inputs screened included degree day accumulation (base 10°C, DD_{Ac}) from the previous late summer starting 1 August; DD_{Ac} current year starting 1 January and 1 May; and average relative humidity (RH), solar radiation (SR), vapor pressure deficit (VPD), pan evaporation (E_{pan}), temperature, and precipitation between 1 May and 31 July of the current year.

The aforementioned timeframes were used because of their relationship to (i) ascocarp development and survival, or (ii) their inclusion of the period of peak fruit susceptibility. For initial evaluations only years between 1986 and 2007 classified as

'Severe' or 'Mild' with respect to cluster disease severity were used, where the definition of 'Severe' was >20% disease severity on clusters, or 'Mild' as <6% disease severity on clusters. In subsequent analyses the full disease severity data set was used and 'Severe' or 'Mild' years were redefined as between >9 and 20%, and <9%, respectively. For the purpose of logistic regression analysis and Recursive Partition Analysis, 'Mild' years were coded as 0 and 'Severe' years were coded as 1. The absolute prediction of a 'Severe' year was adjusted using Receiver operating curve (ROC) analysis to compare sensitivity and specificity of the developed model (27), rather than reporting the raw predicted probability.

Recursive partition analysis (RPA) (11) was used to develop a decision tree for epidemic classification. Recursive partition analysis predicted the probability that a given year would fall into the 'Severe' or 'Mild' categories, based on threshold values of E_{pan} and DD_{Ac} from the late summer of the previous year. The output categories were defined by the percentage of the population within that category (as defined by the previously mentioned thresholds) whose final disease classification was 'Severe' or 'Mild'. There were four final prediction categories for RPA: '100% Severe', '100% Mild', '60% Severe', and '80% Mild'.

For the described logistic regression and RPA, average weekly E_{pan} from 1June to 2 August was used to test how well the models adjusted predictions based on changing in-season weather. To do this, average daily E_{pan} was recalculated on a weekly basis, including all daily data from 1 June and onward for each year (i.e. the daily average E_{pan} calculated 7 June was an average of 7 days, the daily average E_{pan} calculated on 14 June was the average of 14 days, etc.).

Incorporation of acute cold temperature events in model development. In addition to the aforementioned weather parameters, historical weather data was analyzed to see if there was a relationship between cluster disease severity and the

occurrence of acute cold temperature events between 1 and 31 May, as acute cold temperature events have been shown to induce grapevine resistance to powdery mildew and hinder development of nascent powdery mildew colonies (33).

This time frame was chosen as it spans the period of budbreak to cluster emergence, and is the portion of the early growing season not accounted for in the aforementioned analyses for model development. To build a potential "rule-of-thumb" for the effects of acute cold on cluster disease development, the total number of days where the minimum temperature was at or below (i) 10°C and (ii) 5°C was regressed against log transformed cluster disease severity.

Validation of the New York model. Daily weather records from the NOAA benchmark weather station for the years 1978 thru 1985, 2008, and 2009, in combination with observations of fruit disease severity for Geneva, NY were used as a validation data set. Full-season data comprised of the weather parameters employed in model development were used. In addition, average daily E_{pan} was recalculated on a running weekly basis as previously described to divide the growing season into key grapevine phenological stages which are traditional benchmarks in spray regimes (i.e., budbreak, bloom, and fruit set).

Comparison of observed and calculated pan evaporation. Consequences of substituting calculated evapotranspiration (E_{to}) for observed evaporation readings in the absence of a Class A Meterological Evaporation Pan (E_{pan}) were inferred by regressing E_{to} as calculated by the FAO modified Penman-Monteith equation (Table 3.3; Equation 3.1) against observed E_{pan} for the years 1985-2007. For days when key input records were missing, E_{to} was not calculated.

In addition, calculated E_{to} was converted to calculated E_{pan} by the following equation:

$$\mathbf{E_{to}=K_p*E_{pan}} \qquad \textbf{(Equation 3.2)}$$

where K_p is the pan coefficient and is related to wind speed, distance of pan from fetch, and relative humidity (1). In most cases K_p=0.8. As before, linear regression was used to compare observed $E_{pan,}$ to calculated E_{pan}.

A final comparison of calculated evapotranspiration to observed pan evaporation was made using a version of the Penman-Monteith equation (Table 3.3, Equation 3.3), that calculates evaporation (E_o) from an open water surface (16), similar to that of a Class A Meteorological Evaporation Pan.

Table 3.3- Penman-Monteith equation for evapotranspiration and associated weather and location input parameters.

Symbol	Description	Units	Equation or Value
E_{to}	reference evapotranspiration	mm day^{-1}	$E_{to}=(0.408\Delta(R_n-G) + \gamma(900/(T+273)) u_2(e_s-e_a))/ (\Delta+ \gamma(1+0.34 u_2)))$ (**Equation 3.1**)
E_o	Open water evapotranspiration	mm day^{-1}	$E_o=[\Delta(Rn-G)/2.45 +\gamma(u_{2*}(e_s- e_a)]/(\Delta+ \gamma)$ (**Equation 3.3**)
R_n	net radiation at surface	MJ m^{-2} day^{-1}	
G	soil heat flux density	MJ m^{-2} day^{-1}	0
$T_{ave,}$ $T_{max,}$ T_{min}	Daily temperature	°C	Air temperature at 2 m height $T_{ave}=[(T_{max}+T_{min})/2]$
u_2	wind speed	m s^{-1}	At 2 m height
e_s	saturation vapor pressure	kPa	$=[0.6103*e^{((17.27*Tmax)/(Tmax+237.3))} + 0.6103*e^{((17.27*Tmin)/(Tmin+237.3))}]/2$
e_a	actual vapor pressure	kPa	$=[0.6103*e^{((17.27*Tmax)/(Tmax+237.3))*(RHmin/100))} + 0.6103*e^{((17.27*Tmin)/(Tmin+237.3))*(RHmax/100)})]/2$
RH	relative humidity	%	
Δ	slope vapor pressure curve		$=(4098* E_s)/(((T_{max}+237.3)+(T_{min}+237.3))/2)^2$
γ	psychrometric constant	kPa $°C^{-1}$	$=0.00665^3*P$
P	atmospheric pressure	kPa	$=101.3*((293–0.0065*z)/293)^{5.26}$
z	meters above sea level	m	

Monte Carlo Simulation of calculated E_{pan} using historical weather inputs.

In order to predict potential E_{pan}, Monte Carlo simulation was used to calculate E_{to} from Eq. 3.1. Historical weekly average maximum and minimum temperatures, minimum and maximum vapor pressure deficit, wind speed, and net solar radiation were used, setting an 80% tolerance interval for the population with a 95% confidence interval for the values. Evapotranspiration was not converted to calculated E_{pan} (conversion using Eq. 3.2), as E_{to} was more consistent in predicting observed E_{pan}. Historical daily input data from 1985-2007 are summarized in Table 3.4. Soil heat flux and psychometric constant were held constant, and the slope of the vapor pressure curve changed with changing temperature inputs. The simulation was run using an Excel-based spreadsheet, and calculations were based on 5000 runs/combinations. Further detail about the Excel®-based Monte Carlo simulation can be found in Appendix 1.

Table 3.4- Historical minimum and maximum values for weather parameters necessary for calculating evapotranspiration. Daily weather records from 1985 to 2007 for Geneva, NY were used.

	Solar Radiation (MJ m^{-2} day^{-1})		VPD (kPa)		Ave Temp. (°C)		Wind (m/s)	
Week Timeframe	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
6/1-6/7	14.0	23.4	0.23	0.81	12.7	20.7	0.76	2.12
6/8-6/14	13.8	26.2	0.23	0.96	14.6	22.8	0.65	2.22
6/15-6/21	15.1	25.5	0.19	1.01	15.3	24.1	0.63	1.89
6/22-6/28	13.5	27.2	0.29	1.06	16.8	24.4	0.54	2.10
6/29-7/5	15.5	24.8	0.32	0.97	17.2	23.5	0.59	2.02
7/6-7/12	16.5	24.0	0.27	1.06	17.1	25.4	0.85	1.72
7/13-7/19	13.9	25.0	0.24	1.02	18.4	25.2	0.63	1.57
7/20-7/26	14.4	23.5	0.24	0.98	17.9	25.3	0.55	1.77
7/27-8/2	13.0	25.2	0.21	0.95	17.7	24.8	0.63	1.67

Statistical analyses. All statistics performed using JPM Statistical Software (SAS Institute, Inc., Cary, North Carolina, USA), using either the "Screening", "Fit Model" or "Recursive Partitioning" platforms.

RESULTS

Evaluation of existing powdery mildew models using New York data. The UC-Davis Powdery Mildew Risk Index was initiated between 11 and 20 d prior to bloom, and in all cases, did not fall below the high-risk category after CDOY 122 (30 June; 1 to 2 weeks postbloom) (Fig. 3.1 and Table 3.5). In only two of the seven years did the Risk Index have one or more "Low" ratings, and in only three of seven years did it have any "Intermediate" ratings. All years, except for 1985, were classified as 'Severe' both at and after bloom.

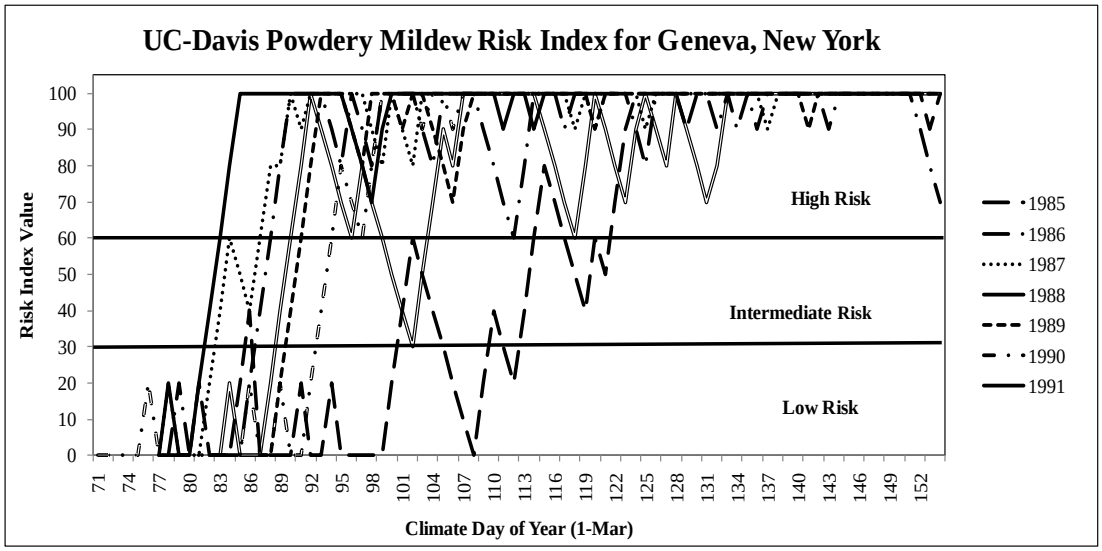

Figure 3.1- University of California-Davis Risk Index results for Geneva, NY. Risk assessment began 10 d after the first rainfall of 2.5 mm coincident with temperatures above 10°C. The Risk Index was not initiated until 3 consecutive days with 6 or more continuous hours of temperatures between 21 and 29.5°C was achieved.

Table 3.5- Output from UC-Davis Powdery Mildew Risk Index for 1985 thru 1991 for Geneva, NY.

Year	Cluster Severity	Budbreak[w]	Bloom[w]	First PM[w,x]	Initial Infection[w,y]	Risk Index Start[w]	Days in Moderate Risk[w]	Days in Low Risk[w]
1985	14.0	64	113	90	77	102	103-105, 110, 113, 118, 119, 121	106-109, 111, 112
1986	48.2	59	103	88	78	88		
1987	8.2	65	102	94	77	84	85, 85	
1988	0.2	70	109	n/a	82	90	100, 101, 103	102
1989	47.7	70	111	98	87	91		
1990	6.4	59	108	94	71	94		
1991	2.9	62	97	91	77	83		

[w] Listed as climate day, starting 1 March.

[x] Recorded as first signs of powdery mildew in the field, as noted by R. Pearson. No observation data was available for 1988 due to a sabbatical leave.

[y] Initial infection as described in the UC-Davis Powdery Mildew Risk Index protocol: Assumption of first signs 10 d post an infection event where 2.5mm rain is followed by 13 h leaf wetness and temperatures between 10 and 27°C after budbreak

[z] Risk Index started when epidemic requirements are satisfied after initial infection as described in the UC-Davis Powdery Mildew Risk Index.

The first spray of the season was initiated by the OiDiag 2.2 expert system between CDOY 89 and 121 (Table 3.6), but the timing of this first spray was not related to subsequent epidemic severity on unsprayed grape clusters. The earliest suggested first spray occurred in 1990, when cluster disease severity reached 6.4%, whereas the latest initiation of the first spray occurred in 1989, a year when cluster severity reached 47.7%.

Table 3.6- Output from the OiDiag 2.2 expert system from 1985 thru 1991 for Geneva, New York, USA.

Year	Cluster Severity	EL 9[w,x]	Prev. Year Disease Rating	Abs of Mean Min Temp[y]	Day of First Spray[w,z]	Total Sprays*
1985	14.0	78	5	20.8	92	7
1986	48.2	73	3	20.6	97	7
1987	8.2	79	5	21.9	95	8
1988	0.2	84	3	23.1	111	4
1989	47.7	84	1	22.8	121	4
1990	6.4	73	5	21.9	89	n/a
1991	2.9	76	3	20.0	99	n/a

[w] Listed as climate day, starting 1 March.
[x] Calculated as 14 d post-budbreak.
[y] Absolute value of the average of the single minimum temperatures from the previous two winters. Winter defined as October-March.
[z] As calculated in the OiDiag 2.2 expert system, available at: www.oidiag.de.vu
* Total sprays using wettable sulfur in the OiDiag 2.2 expert system, between the date of first spray and 31 July.

Development of a New York model. Initial data screening with regression analysis of log transformed cluster severity ratings from 'Mild' and 'Severe' years between 1986 and 2007 against various weather parameters showed that previous late summer degree day accumulation (base 10°C) from 1 Aug to 15 Sept, and current year pan evaporation from 1 June to 31 July, were significantly correlated with cluster disease severity. Degree day accumulation was significantly positively correlated (slope significant at P= 0.02), and explained 35% of the variance in 'Severe' and

'Mild' disease ratings. Pan evaporation was significantly negatively correlated (slope significant at P=0.003), and explained 68% of the variance in 'Severe' and 'Mild' disease ratings. When combined for multiple regression, the resulting model explained 81% of the variance, and an ANOVA of the model was significant at P<0.001; the regression model explained the data better than a fit of the means.

The regression equation relating cluster disease severity to pan evaporation (E_{pan}) and autumn degree day accumulation (DD_{Ac}) was:

$$\mathbf{Log_{(Cluster\ Severity\ M/S\ Only)} = 4.68 - 1.40(E_{pan}) + 0.013\ (DD_{Ac})} \quad \textbf{(Equation 3.3)}$$

While the intercept was not significant (P=0.14), the parameter estimates for pan evaporation and degree day accumulation were statistically different from zero (P=0.0003 and 0.01, respectively).

Subsequent regression including all years of disease data from 1986-2007, the previous year late summer degree day accumulation (base 10°C) from 1 Aug to 15 Sept, and current year pan evaporation from 1 June to 31 July resulted in a model that explained 60% of the variance, and an ANOVA of the model was significant at P<0.0002; the regression model explained the data better than a fit of the means.

The regression equation relating cluster disease severity to pan evaporation (E_{pan}) and autumn degree day accumulation (DD_{Ac}) was:

$$\mathbf{Log_{(Cluster\ Severity\ All\ Years)} = 4.44 - 1.09(E_{pan}) + 0.01\ (DD_{Ac})} \quad \textbf{(Equation 3.4)}$$

While the intercept was not significant (P=0.10), the parameter estimates for pan evaporation and degree day accumulation were statistically different from zero (P=0.0007 and 0.03, respectively).

From this initial screening, E_{pan} from 1 June through 31 July and DD_{Ac} from 1 Aug to 15 Sept were used in all subsequent model development. Logistic regression analysis based years from 1986 to 2007 resulted in a model with an R^2 of 0.31, but the X^2=0.009 indicated that the model was a better fit than mean response alone.

The response equation for the probability of either a 'Mild' or 'Severe' year is below:

Prob('Mild')= 1/[1+e^^-(-0.45+(-0.02*DDAc)+(1.98*Epan))] **(Equation 3.5)**

Prob('Severe')=1-Prob('Mild') **(Equation 3.6)**

Receiver Operating Curve (ROC) had an area under the curve (AUC) of 0.86, and a maximum sensitivity (100%, ability to predict mild years) and specificity (67%, ability to predict severe years), when the predicted probability of a 'Severe' year is 0.29 or higher. This ROC threshold indicated that when a prediction probability for a 'Severe' year was 0.29 or higher, it should be classified as a 'Severe' year. This threshold resulted in 4 false positives (prediction of a 'Severe' year when in fact mild), and no false negatives (prediction of a 'Mild' year when severe) out of the 22 years used in building the model (Table 3.7).

Table 3.7- Verification of the logistic regression model using data from 1986-2007.

Year	Observed Disease Level	Prob('Mild')	Prob('Severe')	Final Classification
1986	Severe	0.40	0.60	Severe
1987	Mild	0.79	0.21	Mild
1988	Mild	0.98	0.02	Mild
1989	Severe	0.19	0.81	Severe
1990	Mild	0.54	0.46	Severe
1991	Mild	0.91	0.09	Mild
1992	Severe	0.05	0.95	Severe
1993	Mild	0.91	0.09	Mild
1994	Severe	0.33	0.67	Severe
1995	Mild	0.94	0.06	Mild
1996	Severe	0.22	0.78	Severe
1997	Severe	0.51	0.49	Severe
1998	Severe	0.70	0.30	Severe
1999	Mild	0.89	0.11	Mild
2000	Mild	0.19	0.81	Severe
2001	Mild	0.73	0.27	Mild
2002	Mild	0.49	0.51	Severe
2003	Severe	0.16	0.84	Severe
2004	Mild	0.23	0.77	Severe
2005	Severe	0.71	0.29	Severe
2006	Severe	0.21	0.79	Severe
2007	Mild	0.92	0.08	Mild

Recursive partition analysis split the data three times (Fig. 3.2), with a resulting R^2=0.61, and an ROC analysis with an AUC of 0.94 for both 'Severe' and 'Mild' years. The first split was by E_{pan}, and divided very dry years from average to wet years. The second split was by autumn temperature accumulation, and the third split divided wet from average years (Fig. 3.2). Verification of the RPA decision scheme with actual disease severity outcomes from 1985-2007 resulted in 19 true 'Severe' or '60% Severe' predictions, 2 false 'Severe' or '60% Severe' predictions, and one prediction of a 'Mild' year when it was in fact a severe year (Table 3.8).

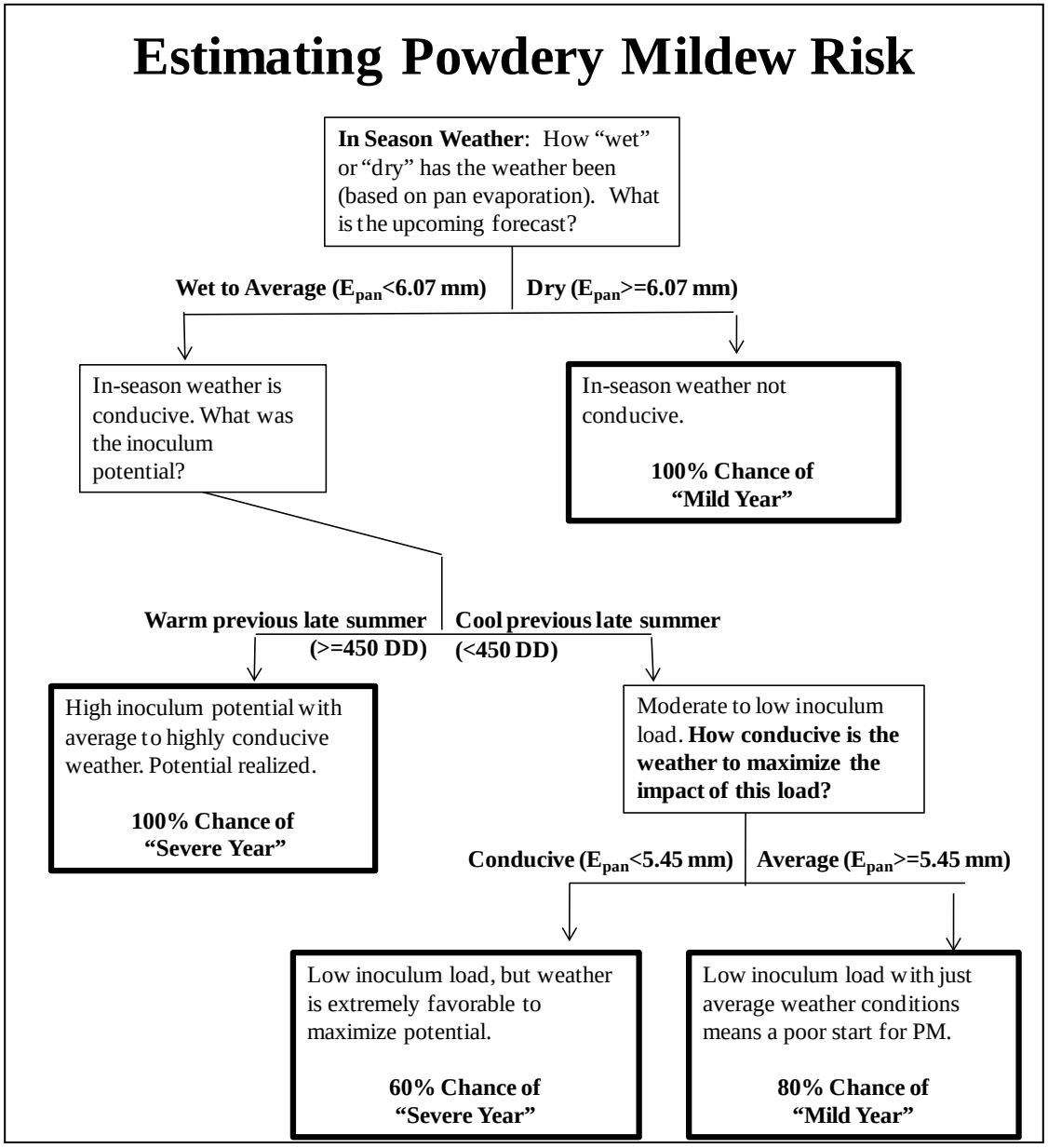

Figure 3.2- Decision scheme for 'Mild' and 'Severe' years for grapevine powdery mildew epidemics derived from recursive partitioning. This scheme defines "threshold" levels of both DD_{Ac} and E_{pan} that provide the greatest influence on epidemic development.

Table 3.8- Verification of the recursive partitioning model using data from 1985-2007.

Year	Observed Disease Level	Final Classification
1986	Severe	60% Severe
1987	Mild	80% Mild
1988	Mild	Mild
1989	Severe	60% Severe
1990	Mild	80% Mild
1991	Mild	Mild
1992	Severe	Severe
1993	Mild	80% Mild
1994	Severe	Severe
1995	Mild	Mild
1996	Severe	Severe
1997	Severe	Severe
1998	Severe	60% Severe
1999	Mild	Mild
2000	Mild	60% Severe
2001	Mild	80% Mild
2002	Mild	Mild
2003	Severe	Severe
2004	Mild	60% Severe
2005	Severe	80% Mild
2006	Severe	Severe
2007	Mild	Mild

Based on in-season weekly E_{pan} averages, comparison of the logistic regression and RPA resulted in very similar week-to-week predictions, and the breakdown of weather inputs to weekly running average segments did not appear to negatively affect models' predictions (Table 3.9). Recursive partition analysis provided better insight into subtle changes in weather during the growing season, whereas logistic regression analysis was less sensitive to these changes.

Table 3.9- Comparing logistic regression and recursive partition for verification using weekly accumulating average for years 1988-2007.

Year	Analysis[x]	6/1-6/7	6/1-6/15	6/1-6/21	6/1-6/30	6/1-7/7	6/1-7/15	6/1-7/21
1988	Log	Mild	Mild	Mild	Mild	Mild	Mild	Mild
	RP*	Mild	Mild	Mild	Mild	Mild	Mild	Mild
1989	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	60% S	60% S	60% S	60% S	60% S	60% S	60% S
1990	Log	Mild	Severe	Mild	Severe	Severe	Severe	Severe
	RP*	Mild	80% M	80% M	80% M	80% M	80% M	80% M
1991	Log	Severe	Mild	Mild	Mild	Mild	Mild	Mild
	RP*	80% M	Mild	Mild	Mild	Mild	Mild	Mild
1992	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	Severe	Severe	Severe	Severe	Severe	Severe	Severe
1993	Log	Severe	Mild	Mild	Mild	Mild	Mild	Mild
	RP*	60% S	60% S	60% S	60% S	60% S	80% M	80% M
1994	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	Severe	Severe	Mild	Severe	Severe	Severe	Severe
1995	Log	Mild	Mild	Mild	Mild	Mild	Mild	Mild
	RP*	Mild	Mild	Mild	Mild	Mild	Mild	Mild
1996	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	Mild	Severe	Severe	Severe	Severe	Severe	Severe
1997	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	Severe	Severe	Severe	Mild	Severe	Severe	Severe

[x]RP=Recursive Partitioning, Log=Logistic regression. Logistic regression output was adjusted using the ROC cut off where any year above a 0.29 probability of 'Severe' is severe.
*M=Mild, S=Severe

Table 3.9*, continued*

Year	Analysis[x]	6/1-6/7	6/1-6/15	6/1-6/21	6/1-6/30	6/1-7/7	6/1-7/15	6/1-7/21
1998	Log	Mild	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	80% M	60% S	60% S	60% S	60% S	60% S	80% M
1999	Log	Severe	Mild	Mild	Mild	Mild	Mild	Mild
	RP*	60% S	Mild	Mild	Mild	Mild	Mild	Mild
2000	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	60% S	60% S	60% S	60% S	60% S	60% S	60% S
2001	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	60% S	60% S	80% M	80% M	80% M	80% M	80% M
2002	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	Severe	Severe	Severe	Severe	Severe	Severe	Mild
2003	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	Severe	Severe	Severe	Severe	Severe	Severe	Severe
2004	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	60% S	60% S	80% M	80% M	80% M	80% M	80% M
2005	Log	Severe	Mild	Severe	Mild	Mild	Mild	Mild
	RP*	80% M	Mild	80% M	Mild	Mild	Mild	Mild
2006	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
	RP*	Severe	Severe	Severe	Severe	Severe	Severe	Severe
2007	Log	Severe	Mild	Mild	Mild	Mild	Mild	Mild
	RP*	60% S	Mild	Mild	Mild	Mild	Mild	Mild

[x]RP=Recursive Partitioning, Log=Logistic regression. Logistic regression output was adjusted using the ROC cut off where any year above a 0.29 probability of 'Severe' is Severe.
*M=Mild, S=Severe

Incorporation of acute cold temperature events in model development. There was no significant relationship between historical acute cold temperature events from 1 to 31 May and cluster disease severity. The 95% confidence interval for the number of nights falling below 10°C from 1 May to 31 May was 19.2-23.9 days, and was 6.8-10.6 nights for the 5°C threshold. Regression analyses comparing log cluster disease severity to number of nights falling below 10°C and 5°C did not have slopes significantly different from zero (P=0.58 and 0.90, respectively). Acute cold temperature events were not included in further model refinement.

Validation of the New York model. Validation of the logistic regression model resulted in one instance of misclassification, which occurred in 1980. The model predicted a 'Severe' year, when it was a mild year for powdery mildew (Table 3.10).

Table 3.10- Validation of Logistic Regression Model using data from 1978-1985, and 2008-2009.

Year	Observed Disease Level	Prob('Mild')	Prob('Severe')	Predicted Disease Level[x]
1978	Mild	0.74	0.26	Mild
1979	Severe	0.44	0.56	Severe
1980	Mild	0.43	0.57	Severe
1981	Severe	0.25	0.75	Severe
1982	n/a	0.34	0.66	Severe
1983	Mild	0.98	0.02	Mild
1984	Severe	0.37	0.63	Severe
1985	Severe	0.46	0.54	Severe
2008	Severe	0.27	0.73	Severe
2009	Severe	0.53	0.47	Severe

Validation of the RPA model based on the 1978-1985, and 2008-2009 dataset resulted in 5 years with disease misclassifications (Table 3.11). The first two misclassifications occurred in 1979 and 1985, when the model predicted an 80%

probability of a ‘Mild’ year both when there they were, in fact, severe years. An additional misclassification was in 1984, when the model predicted a ‘Mild’ year and it was a severe year. Two additional misclassifications occurred in 1980 and 1982, when the model predicted a 60% probability of a ‘Severe’ year for both when they were, in fact, mild.

Table 3.11- Validation of the recursive partitioning model using data from 1978-1985, and 2008-2009.

Year	Observed Disease Level	Final Classification
1978	Mild	80% Mild
1979	Severe	80% Mild
1980	Mild	60% Severe
1981	Severe	Severe
1982	n/a	Mild
1983	Mild	60% Severe
1984	Severe	Mild
1985	Severe	80% Mild
2008	Severe	Severe
2009	Severe	60% Severe

A week-by-week comparison of the logistic regression and RPA models for the validations years mentioned above showed that the weeks immediately surrounding bloom had the largest influence of final cluster severity outcome, and the encompassing weeks acted as modifiers to these predictions. As with the verification data, potential reasons for final disease misclassification was apparent by viewing the timeframes where the model was changing from one severity classification category to the next. The year with the largest discrepancy between the logistic regression and RPA models was 1979, where the logistic regression model classified all weeks as ‘Severe’, whereas the RPA model classified all weeks as ‘Mild’ or ‘80% Mild’.

Table 3.12- Comparing logistic regression and recursive partition for model validation using weekly accumulating average for years 1978-1985, and 2008-2009.

Year	Observed Disease	Analysis[x]	6/1-6/7	6/1-6/15	6/1-6/21	6/1-6/30	6/1-7/7	6/1-7/15	6/1-7/21
1978	Mild	Log	Severe	Severe	Severe	Severe	Severe	Mild	Mild
		RP*	80% M	60% S	80% M	80% M	80% M	80% M	Mild
1979	Severe	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
		RP*	80% M	80% M	Mild	80% M	80% M	80% M	80% M
1980	Mild	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
		RP*	80% M	60% S	60% S	60% S	60% S	60% S	60% S
1981	Severe	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
		RP*	Severe	Severe	Severe	Severe	Severe	Severe	Mild
1982	n/a	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
		RP*	60% S	60% S	60% S	60% S	60% S	60% S	60% S
1983	Mild	Log	Severe	Mild	Mild	Mild	Mild	Mild	Mild
		RP*	60% S	60% S	80% M	80% M	Mild	Mild	Mild
1984	Severe	Log	Severe	Mild	Severe	Severe	Severe	Severe	Severe
		RP*	Severe	Mild	Mild	Mild	Severe	Severe	Mild
1985	Severe	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
		RP*	60% S	60% S	60% S	60% S	60% S	60% S	60% S
2008	Severe	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
		RP*	Severe	Mild	Severe	Severe	Severe	Severe	Severe
2009	Severe	Log	Severe	Severe	Severe	Severe	Severe	Severe	Severe
		RP*	60% S	60% S	60% S	60% S	60% S	60% S	60% S

[x]RP= Recursive partitioning model, Log=Logistic regression model. Logistic regression model output was adjusted using the ROC cut off of classifying anything listed as a 0.29 probability of 'Severe' or higher as 'Severe'.

*M=Mild, S=Severe

Comparing observed and calculated pan evaporation. Calculated E_{to} was moderately to highly correlated with observed E_{pan} (Table 3.13). Use of calculated E_{to} to accurately predict observed E_{pan} was not consistent from year to year. For 14 of the 23 years, calculated E_{to} significantly overestimated E_{pan} (slope < 1). One such year, 2004, had a failure in the solar radiation sensor, thus did not allow calculation of E_{to} from existing weather data. In most years (12 of 23), the intercept was not significantly different from zero.

Table 3.13- Resulting regression parameters from comparing calculated E_{to} (Eq. 3.1) to actual E_{pan}.

Year	R^2	Slope (p-value)[x]	Intercept (p-value)[y]
1985	0.87	1.03 (0.36)	**-0.45 (0.02)**
1986	0.81	0.98 (0.61)	-0.10 (0.61)
1987	0.79	0.99 (0.81)	0.01 (0.97)
1988	0.80	1.06 (0.16)	-0.314 (0.22)
1989	0.80	**0.92 (0.03)**	-0.29 (0.16)
1990	0.78	0.95 (0.21)	-0.30 (0.17)
1991	0.59	**0.88 (0.05)**	0.41 (0.26)
1992	0.61	**0.86 (0.02)**	0.03 (0.91)
1993	0.75	1.00 (1.00)	-0.27 (0.31)
1994	0.57	**0.79 (<0.01)**	**0.60 (0.05)**
1995	0.60	0.95 (0.46)	0.36 (0.34)
1996	0.56	**0.83 (0.01)**	**0.71 (0.02)**
1997	0.45	**0.80 (0.01)**	**1.08 (0.01)**
1998	0.48	**0.80 (<0.01)**	**0.80 (0.05)**
1999	0.59	0.97 (0.65)	0.12 (0.74)
2000	0.44	**0.77 (0.01)**	**0.89 (0.04)**
2001	0.61	0.95 (0.43)	0.37 (0.30)
2002	0.38	**0.52 (<0.01)**	**2.27 (<0.01)**
2003	0.23	**0.53 (<0.01)**	**2.01 (<0.01)**
2004	0.04	**0.20 (<0.01)**	**3.27 (<0.01)**
2005	0.15	**0.51 (<0.01)**	**2.37 (<0.01)**
2006	0.55	**0.85 (0.03)**	0.49 (0.19)
2007	0.47	**0.81 (0.01)**	**1.03 (0.02)**

[x] Testing that slope is different than 1 (2-tailed).
[y] Testing that the intercept is different than 0 (2-tailed).

Calculated E_{pan} was also moderately to highly correlated with observed E_{pan} (Table 3.14). For 10 of the 23 years, calculated E_{pan} significantly underestimated observed E_{pan} (slope > 1). For 4 of the 23 years, calculated E_{pan} significantly overestimated E_{pan} (slope <1). Only in recent years (post-2002) has calculated E_{pan} been overestimating actual E_{pan}. In most years (12 of 23), the intercept was not significantly different from zero. However, in more recent years (post-2002), regression intercepts were more consistently different from zero.

Table 3.14- Resulting regression parameters from comparing calculated E_{pan} (Eq. 3.2) to actual E_{pan}.

Year	R^2	Slope (p-value)[x]	Intercept (p-value)[y]
1985	0.87	**1.29 (<0.01)**	**-0.45 (0.02)**
1986	0.81	**1.22 (<0.01)**	-0.10 (0.61)
1987	0.79	**1.23 (<0.01)**	0.01 (0.97)
1988	0.80	**1.33 (<0.01)**	-0.31 (.22)
1989	0.80	**1.15 (<0.01)**	-0.29 (0.16)
1990	0.78	**1.18 (<0.01)**	-0.30 (0.17)
1991	0.59	1.10 (0.16)	0.41 (.26)
1992	0.61	1.08 (0.28)	0.03 (0.91)
1993	0.75	**1.24 (<0.01)**	-0.27 (0.31)
1994	0.57	0.99 (0.89)	0.60 (0.05)
1995	0.60	**1.19 (0.03)**	0.36 (0.34)
1996	0.56	1.03 (0.69)	**0.71 (0.02)**
1997	0.45	1.00 (0.97)	**1.08 (.01)**
1998	0.48	1.00 (0.99)	**0.80 (0.05)**
1999	0.59	**1.21 (0.01)**	0.13 (0.74)
2000	0.44	0.97 (0.76)	**0.89 (0.04)**
2001	0.61	**1.19 (0.02)**	0.37 (0.30)
2002	0.38	**0.65 (<0.01)**	**2.27 (<0.01)**
2003	0.23	**0.66 (<0.01)**	**2.01 (<0.01)**
2004	0.04	**0.25 (<0.01)**	**3.27 (<0.01)**
2005	0.16	**0.63 (<0.01)**	**2.37 (<0.01)**
2006	0.55	1.08 (0.38)	0.49 (0.19)
2007	0.48	1.02 (0.83)	**1.04 (0.02)**

[x] Testing that slope is different than 1 (2-tailed).
[y] Testing that the intercept is different than 0 (2-tailed).

Calculated E_o was moderately to highly correlated with observed E_{pan} (Table 3.15). For 14 of the 23 years, calculated E_o significantly overestimated observed E_{pan} (slope < 1). The greatest concentration of calculated E_o overestimating actual E_{pan} was been in recent years (post-2002). In most years (14 of 23), the intercept was not significantly different from zero.

Table 3.15- Resulting regression parameters from comparing calculated E_{to} (Eq. 3.3) to actual E_{pan}.

Year	R^2	Slope (p-value)[x]	Intercept (p-value)[y]
1985	0.86	1.00 (1.00)	-0.31 (0.09)
1986	0.81	0.95 (0.19)	-0.04 (0.86)
1987	0.78	0.97 (0.55)	-0.02 (0.93)
1988	0.76	1.06 (0.25)	-0.19 (0.51)
1989	0.79	**0.9 (0.01)**	-0.29 (0.17)
1990	0.78	0.93 (0.07)	-0.27 (0.22)
1991	0.57	**0.86 (0.02)**	0.46 (0.23)
1992	0.61	**0.84 (0.01)**	-0.003 (0.99)
1993	0.75	0.97 (0.48)	-0.24 (0.37)
1994	0.56	**0.76 (<0.01)**	**0.60 (0.05)**
1995	0.59	0.93 (0.30)	0.29 (0.44)
1996	0.57	**0.81 (<0.01)**	0.56 (0.07)
1997	0.45	**0.77 (<0.01)**	**1.08 (0.01)**
1998	0.49	**0.77 (<0.01)**	**0.92 (0.02)**
1999	0.58	0.95 (0.48)	0.24 (0.55)
2000	0.44	**0.75 (<0.01)**	**0.99 (0.02)**
2001	0.59	0.94 (0.35)	0.47 (0.20)
2002	0.38	**0.50 (<0.01)**	**2.34 (<0.01)**
2003	0.30	**0.49 (<0.01)**	**2.18 (<0.01)**
2004	0.04	**0.17 (<0.01)**	**3.37 (<0.01)**
2005	0.13	**0.45 (<0.01)**	**2.66 (<0.01)**
2006	0.56	**0.86 (0.04)**	0.41 (0.28)
2007	0.47	**0.80 (0.01)**	**0.11 (0.02)**

[x] Testing that slope is different than 1 (2-tailed).
[y] Testing that the intercept is different than 0 (2-tailed).

Monte Carlo simulation of calculated E_{pan} with historical weather inputs. Weekly output of the Monte Carlo simulation based on historical weather data inputs included the range of E_{to} values calculated from 5000 possible combinations of the input parameters for that given week. Historically, potentially high E_{to} values that would limit powdery mildew spread according to the rules in Fig. 3.2 were most likely to occur immediately following bloom (22 June-28 June) (Table 3.16). Low E_{to} values that would be conducive for powdery mildew development were most likely to occur the first week of June, and between 4-6 weeks post-bloom. Weather was generally very conducive (low E_{to}) in New York during the time frame from 2 weeks pre-bloom to 6 weeks post-bloom, when fruit were at their peak susceptibility.

Table 3.16- Output of the Monte Carlo simulation for E_{to} using historical average weather inputs from Table 3.4. Output was elastic and changed slightly due to randomization of parameters, but probability levels and central ranges remained relatively unchanged.

Week[x]	Mean E_{to} (mm)	90% Central Range (mm)	$P(E_{to}<5.46mm)$	$P(E_{to}>6.07mm)$
6/1-6/7	4.45	3.46-5.46	0.96	0.00
6/8-6/14	4.91	3.61-6.23	0.72	0.09
6/15-6/21	5.02	3.89-6.22	0.70	0.09
6/22-6/28	5.20	3.76-6.69	0.60	0.20
6/29-7/5	5.07	4.09-6.13	0.68	0.07
7/6-7/12	5.21	4.27-6.12	0.67	0.07
7/13-7/19	5.00	3.79-6.20	0.69	0.10
7/20-7/26	4.86	3.85-5.89	0.80	0.02
7/27-8/2	4.80	3.52-6.13	0.74	0.07

[x] Dates denoted as month/day.

DISCUSSION

Models that are driven by temperature data or initiated after the first signs of disease are inconsistent in their description of grapevine powdery mildew epidemics in New York. To develop a forecasting model for New York, a more robust input parameter, or multiple weather parameters, were needed to accurately describe the relationship between powdery mildew development and associated weather. One such parameter, $E_{pan,}$ incorporated the major factors that influence development of *E. necator* (temperature, VPD, SR, and the modifying effects of wind) into a single integrative and measurable unit, which appeared to be suitable to use in a climate where weather extremes during the peak of epidemic progress do not reach the values necessary to drastically decrease epidemic rates.

Existing Models. The UC-Davis Powdery Mildew Risk Index was designed to time various fungicide applications based on temperature favorability for powdery mildew growth, where high temperatures serve as a limiting factor on epidemic development. However, the model is not initiated until the first signs of powdery mildew were observed in the field. In a Mediterranean climate, this may be an acceptable practice, as limiting high temperatures can suppress epidemics. However, in climates such as that in New York, limiting high temperatures occur infrequently, if ever, during the growing season. As seen in Fig. 3.1, once the UC-Davis Risk Index was initiated, it rarely went below the "High Risk" zone in Geneva, NY. Delaying a spray program in New York until the first signs of disease are present would leave fruit that are in a highly susceptible stage of development exposed to possible infection. While the UC-Davis Risk Index may be well suited for Mediterranean climates, the underlying assumptions for model initiation and output may be unsuitable for cooler, humid climates like that of New York State.

The OiDiag 2.2 expert system, based on weather inputs from a climate (Weinsburg, Germany) similar to NY, incorporated a prediction of potential overwintering inoculum load, by incorporating both the previous season inoculum load and overwintering conditions. This system, however, is based on the assumption that flag shoots are the predominate source of primary inoculum, and the biological rationale for the effect of winter temperature on timing of the first spray is presumably related to the winter-hardiness of grapevine buds infected with powdery mildew (34). In New York State, winter temperatures invariably reach the extreme obviating survival of infected buds.

Thus the drawback to the OiDiag 2.2 expert system in New York State is that it underestimates primary inoculum availability on the basis of the region's low winter temperatures while failing to account for variable levels of cleistothecia production and survival. For example, in 1989, a very severe year for powdery mildew infection on fruit, the Oidiag 2.2 expert system did not call for a first spray until 29 June, well after bloom; this was despite extremely favorable conditions calculated by the in-season index for 3 June, onward, where the risk index value was > 75. Despite these drawbacks to related to the timing of the first spray, the calculation index for in-season weather did relate to weather favorability for powdery mildew development. For example, in severe years, such as 1986, there were a total of 7 calculated sprays from the timing of the first spray to 31 July, whereas in 1988, a very mild year for powdery mildew, there were only 4 such sprays. Even when the spray timing calculations are calibrated to start on the same time for these two years (23 May), the OiDiag 2.2 index suggest a total of 8 sprays for 1986 and 6 sprays for 1988. Incorporating relative humidity into disease prediction, in addition to temperature, appeared to enhance this particular model's ability assess changing conditions that would directly influence powdery mildew growth and development.

Development of a New York grapevine powdery mildew model. In the process of developing a weather-driven powdery mildew risk assessment model for New York State, two main timeframes for weather analysis and the corresponding stage in the powdery mildew life cycle were the likely driving factors for prediction of fruit infection: the previous late summer, during which temperatures influence cleistothecia formation, and the period from prebloom to fruit set, which correspond to the period of secondary inoculum production and fruit susceptibility. This isn't surprising, as other models, particularly for the Fusarium Head Blight of wheat pathosystem, also routinely require similar inputs regarding pathogen lifecycle for successful epidemic prediction (13).

Weather analysis revealed that pan evaporation was a predominant factor that negatively correlated with the outcome of final disease severity on grape clusters. As mentioned previously, E_{pan} is an integrative single parameter influenced by solar radiation, wind, temperature, atmospheric humidity and pressure. Past studies have shown that viticultural practices such as leaf removal can increase vapor pressure deficit within the removal zone, increasing evaporation potential and thus reducing disease (15). Increased exposure to UV light also directly kill powdery mildew colonies, and also raise the surface temperature of exposed grapevine leaves to a level lethal to powdery mildew (4-6). In addition, a previous report by Aust *et al.* (3) suggests that increased temperature and decreased relative humidity within a canopy reduces powdery mildew infection, and they attribute this not only to the direct effects on the pathogen, but also as a result of a more rapid aging of leaf tissue due to abiotic stress. However, with a focus on the ability to *predict* powdery mildew fruit severity with predicted weather inputs, the use of E_{pan} appeared to be limited. Evapotranspiration, on the other hand, can be calculated from various predictable weather components and is a reliable substitution for E_{pan}. However, a major

component of the E_{to} calculation is solar radiation. Solar radiation can be modeled, but with some degree of difficulty. However, the effect of solar radiation on leaf transpiration is related to the intensity of the radiation, with modification by the ability for air movement to cool the leaf surface temperature (24). Solar radiation also directly influences powdery mildew development, as recent studies have demonstrated significant effects of UV exposure and associated increase in surface temperature on e grapevine canopy disease severity; increased solar radiation results in decreased powdery mildew survival on exposed leaves (4, 6).

Other analyses relating weather data to foliar and fruit disease severity and incidence have also confirmed that late-summer heat accumulation positively correlates with following year disease levels. Other authors attribute this to increased favorability for cleistothecia formation (30). The timeframe suggested here, 1 August through 15 September, closely relates to the time when growers restrict powdery mildew fungicide applications for reduction of fungicide residues on fruit as they approach the preharvest interval for fungicides. Overall, however, it appears that while primary inoculum is important in grape powdery mildew epidemics, in-season weather is still driving the epidemic, which is in agreement with past studies (9).

Determination of factors that influence the severity of a powdery mildew epidemic in New York State is one task, but the adaptation of that information into a useful tool for management decisions is another. Of the two models derived from (i) logistic regression, and (ii) RPA, the latter appeared to be more useful for in-season risk forecasts. However, this may have been due to the ROC sensitivity analysis used to define thresholds for 'Severe' or 'Mild' predictions and the application of the various models with weekly average weather data when it was originally derived from seasonal average data. Of greater interest was the changing of models' output over the course of the season, seen in Tables 3.9 and 3.12. It appeared that E_{pan} trends, up

through bloom, were the major driving factors for whether or not clusters became severely infected, and subsequent conditions after bloom only moderated the level of severity. For example, 1998 was classified as a severe year for powdery mildew (9.35% cluster severity), but it started and ended the season with a 'Mild' prediction (based on RPA), and the middle of the season was considered 'Severe'. It was one of the years that fell into an 'Intermediate' category, but was classified as 'Severe' to be conservative, as a false prediction of a 'Severe' year is better than a false prediction of a 'Mild' year from a production standpoint.

The analysis of the impact on acute cold weather events on cluster disease severity showed that early season acute cold events did not appear to influence the degree of fruit infection severity later in the growing season. However, the lack of a relationship is likely due to the consistent annual occurrence of acute cold temperature events in Geneva, NY. The lack of variability in annual acute cold temperature events in Geneva, NY means that such events are equally influencing annual epidemics, and are therefore not likely contributing to the annual variation if fruit disease severity. If adapting this model to other climate locations where the occurrence of acute cold temperature events are more variable, their impact on the development of early-season foliar epidemics will likely be more important as it would influence the amount of inoculum available for subsequent fruit infection.

In the validation process, both models also described epidemics relatively well, although disease data from pre-1985 is limited and vague due. However, there were some inconsistencies. Among the validation years for the RPA model, 1984 and 1985 were classified as 'Mild' and '80% Mild', respectively, though observed data indicated that they were 'Severe' years. However, in 1985 the predicted weekly ratings were '60% Severe' for beginning of the growing season, but a substantial increase in E_{pan} during the last week of July resulted in a large increase in the E_{pan}

average, pulling it into the '80% Mild' prediction category (data not shown). The 1984 anomaly is not as clear cut: 1984 was an exceptionally dry year right around bloom, but the first week of June, and the first half of July had severity ratings classifying it as 'Severe'. The likely scenario is that the abundance of overwintering primary inoculum (it was a warm autumn in 1983 with over 500DD units accumulating) was able to establish quickly in the first week of June, and while it dry for a few weeks later, already established infections were able to maximize growing potential when E_{pan} decreased in the month of July, before finally drying out at the end of July.

On the use of E_{pan} in disease forecasting. One drawback to using E_{pan} as a model input is the level of maintenance needed to keep a Class A Meteorological Pan functioning properly. In addition, while extensive pan evaporation networks exist around the globe, particularly in arid climates where production horticulture relies on irrigation, quality and consistent data is not easy to find. To get around this, many meteorologists model E_{pan} from calculated E_{to}. For Geneva, New York, comparisons between observed E_{pan}, calculated E_{to}, calculated $E_{pan,}$ and calculated E_o showed that calculated E_{to} and calculated E_o may be more suitable calculated estimates in place of observed E_{pan} rather than the calculated E_{pan}. This is especially true in more recent years, as calculated E_{pan} has been consistently underestimating actual E_{pan}. Because of this underestimation, calibrating E_{to} using Eq. 3.2 seems unnecessary for Geneva, New York. Both calculated E_{to}, modified to adjust for transpiration resistance in a plant, and calculated E_o, used to estimate evaporation from an open surface, do not appear to produce drastically difference results in output, and both appear to be suitable and interchangeable for this purpose. An argument can be made that since E_{to} and E_o are similar in output, E_o should be used. This rationale is based on at the effects of water demands on a fungus, and not a plant, and that transpirational resistance for a fungus,

especially a powdery mildew that grows wholly external to the plant, is better suited to be modeled like an open body of water. The counter argument can also be made that the FAO modified Penman-Monteith equation for calculated E_{to} was designed to standardize the calculation process, and that using it to estimate E_{pan} may prove to be a more translational approach that is already designed to meet international standards.

Another shortcoming to using observed E_{pan}, is that it has to be measured before it can be used in either of the proposed models. Knowledge on how current conditions influence powdery mildew development is important, but the ability to forecast how environmental conditions will change is useful. Since E_{to} is based on calculations that use weather parameters that have varying degrees of predictability, it in theory can then be predicted, and ultimately, then E_{pan} can be indirectly predicted.

Prediction of E_{to} with Monte Carlo simulation based on historical weather inputs (i) demonstrated that E_{to} can potentially be forecasted even with limited real-time weather data, and (ii) provided valuable insight as to when the conditions that are modifying powdery mildew epidemics are likely to occur. As seen in Table 3.16, the probability of high E_{to} (>6.07mm), where the epidemic would be limited, was greatest at the time immediately around the average date of bloom in New York. To refine E_{to} prediction, forecasted temperature can easily be substituted for the historical data, as temperature can be reliably predicted out to several days. This would improve the E_{to} output and allow for a better estimation and prediction of potential E_{to}, which can be used in management decisions for powdery mildew control.

On a long-term scale, use of E_{pan} rather than temperature, as a means to forecast powdery mildew epidemics may prove useful in light of recent concerns about global climate change. One component of this reported climate change is a phenomenon called “global dimming” describing the steady decline in global evapotranspiration, attributed to the build-up of atmospheric particulates that result in

a reduction in solar radiation reaching the Earth's surface. The steady decline of observed evapotranspiration, even in light of increased global temperatures, could create more favorable environments for powdery mildew development, especially in areas where high temperatures would normally limit growth (as the lethality of high temperatures to powdery mildew colonies may be due to hyphal dehydration). In addition, the reduction of solar radiation may also favor pathogen development (6). Further studies into the interactions between temperature and moisture stress on powdery mildew may provide powerful insight as to how global climate change will impact powdery mildew epidemic development.

Closing remarks. The grapevine powdery mildew model presented here is less complex than other models within this pathosystem (7, 9, 35), and to other powdery mildew pathosystems (37). Most of these models include host development sub-models, which are especially crucial for determining disease development on foliage as the season progresses. However, with the development of ontogenic resistance in grape berries, and the subsequent limited window for fruit infection, integration of a host development model was unnecessary. While the mechanistic approach to modeling is elegant, with an almost limitless number of subcomponents (both of known and unknown values), the current empirical approach to powdery mildew forecasting was favored because of its simplicity and ease of use. This approach is a common technique for plant disease forecasting (12), especially in combination with field derived experiments.

In conclusion, this approach to prediction of powdery mildew risk severity on grape clusters has the potential to be easily integrated into a web interface, and can link real-time weather forecasts for a given region in order to calculate forecasted E_{to}. While less responsive to hourly or daily changes to environmental conditions, this model, which operates using weekly averages, provides a more robust computation of

relative favorability for powdery mildew development. The prediction output, however, must be considered and modified based on site-specific knowledge of a vineyard such as existing disease pressure, susceptibility of the variety grown, and cultural practices that may influence pathogen development and spread. While future studies can improve E_{pan} forecasting and relate interior canopy E_{to} to observed E_{pan}, this study provides the key foundation blocks for building a risk forecasting system for powdery mildew fruit infection in New York,

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GENERAL CONCLUSIONS

The key in the development of an optimal disease management program is a strong knowledge base of the pathogen. The control of grapevine powdery mildew is no exception. Factors that influence the outcome of an epidemic are diverse and often interconnected. From the host side, species differences in resistance to powdery mildew, phenological differences in development based on winter chilling (11), grapevine health and nutrition status, and trellis and canopy management strategies (6, 23), all contribute to either enhanced or diminished *Erysiphe necator* growth. From the pathogen side, sexual recombination (10), overwintering capabilities (7, 9, 13, 14, 16, 18), and the genetic evolution of fungicide resistance (22) all directly affect the pathogen's ability to successfully establish year after year. Finally, environmental factors can affect both the pathogen and host, and the subsequent disease development can be influenced by precipitation, cloud coverage, humidity, wind speed, solar radiation and temperature (1-5, 8, 12, 15-17, 19, 21).

The research presented within this dissertation addressed the effects of overwintering conditions on the timing of arrival of primary inoculum, the impact and influence of the environment on initial epidemic development, and the macroscale environmental patterns that historically correlate with powdery mildew epidemics in the Northeastern United States. These three studies, though seemingly disjointed in nature, provide key pieces of data formerly missing in the knowledge base on the epidemiology of grapevine powdery mildew. As with any investigation, however, new answers are a springboard for new questions. The following paragraphs outline some potential 'next steps' in the investigation of grapevine powdery mildew epidemics.

In Chapter One, we learned that cleistothecia dehiscence is related autumn to heat-unit accumulation and spring wetting events, and can occur prior to budbreak of *V. vinifera*. This early release, however is not surprising considering *E. necator* co-evolved with different species of *Vitis.* These species, particularly *V. riparia* tend to progress through phenological development at a faster rate. But it is important to understand how this timing relates to and influences the establishment of powdery mildew epidemics in any given year. In years where winter and early spring temperatures are warm, and there are frequent moisture events above 2.5 mm, the primary inoculum supply could be significantly depleted prior to the emergence of susceptible host tissue.

Further investigations showed that the severity of grapevine powdery mildew epidemics on fruit is more dependent upon in-season weather than the absolute quantity of primary inoculum, a classic example of polycyclic disease development (20). Information on the amount of primary inoculum that survives into the growing season (Chapter One), or the fate of that inoculum in the very early stages of disease establishment (Chapter Two), is important, but is not a final determining factor in the levels of fruit infection, especially in years where the disease outcomes are in the extremes. To determine the importance of primary inoculum in this pathosystem, additional controlled environment studies are necessary. One experiment could involve the description of epidemic progress on potted vines where (i) environment and timing of arrival of primary inoculum are held constant, but quantity of primary inoculum is varied; (ii) environment and quantity of primary inoculum is held constant, but the timing of arrival is varied; or (iii) timing of arrival and quantity of primary inoculum are held constant and major environmental influences like temperature, atmospheric moisture, and wind speed are varied (set at the developmental limits of the pathogen). Experiments of this nature have been done in

the field, but only to a small extent under precisely controlled conditions. Larger-scale controlled environment studies would allow for more precise analyses and breakdown of variables that affect epidemic progress without confounding factors such as the variable weather conditions or background disease levels. Comparisons between experiments would also provide additional supporting data for the relative importance of primary and secondary inoculum on grapevine powdery mildew epidemics.

The effects of acute cold (<8°C) temperature events on powdery mildew development and *V. vinifera* susceptibility were investigated in Chapter Two. Previous investigations on temperature effects on powdery mildew development have focused predominately on development that occurred when temperatures were in the "optimum" range, or when temperatures were at the upper limits for powdery mildew development. Investigations presented here on acute low temperature events, however, showed that the low temperature range in powdery mildew development is not to be ignored. Low temperatures not only induced a transient resistance in normally susceptible leaf tissue, they also significantly damaged existing nascent colonies via hyphal mortality. Ambient air temperature measurements used in powdery mildew modeling may be deceiving; radiational cooling of the leaf surface results in temperatures that can be several degrees cooler than the surrounding air and in some cases leaf surface temperature may drop below freezing. Such temperature events can occur in viticultural regions across the globe, from desert climates like those in the Riverland of South Australia, to cool climates like those in the Finger Lakes of New York State.

The next step in the investigation into cold-induced disease resistance for *Vitis* would be to discern whether or not this induced resistance is a common theme in the *Vitis* genera, or specific to certain species. Do species considered cold tolerant (i.e. *V. riparia*) respond to acute cold stress in the same manner that less tolerant species (i.e.

V. vinifera) do? Would acute cold exposure induce a resistance reaction in cold tolerant *Vitis* species, or would a low temperature preference result in stimulated growth that would increase the amount of susceptible tissue and result in the appearance of enhanced susceptibility? I would hypothesize the latter and suggest that the cold-induced disease resistance is a phenomenon seen in warm-climate (cold-intolerant) *Vitis* species. At a more basic level, a genetic or cellular investigation as to why a stress such as acute cold exposure would induce resistance to an obligate biotrophic pathogen may provide key insights to this highly evolved relationship between pathogen and host. How is a response to cold stress related to other stress responses in *Vitis*? More importantly, can we use this knowledge to create or improve upon management strategies?

Understanding the biology of *E. necator* is important for the advancement of science, but of practical importance to grape production is the use of this biological understanding to maximize the impact of disease management strategies. A powdery mildew risk assessment model for New York State was presented in Chapter Three. Mining historical weather data for conditions that influenced the development of powdery mildew on grape clusters revealed that two key stages in *E. necator*'s life cycle, and the related environmental influences, are what correlate with disease severity. These two stages, the development of overwintering cleistothecia which provides the base levels of inoculum the following spring, and the rapid secondary reproduction that occurs during the period of peak cluster susceptibility, are what drive epidemic development and the risk for severe powdery mildew infection on grape berries. While epidemic development and the modeling of epidemics can be seemingly complex, what was presented in Chapter Three were two relatively simple models that can aid in management decisions, without the burden of additional submodels. Its simple design and presentation was intentional, to increase the

likelihood it will be adopted and modified for educational and practical uses. Simplicity allows for adjustment and personalization without loss of reliability and functionality.

The next step in the adoption of the New York Powdery Mildew Risk Assessment model presented in Chapter Three would be to integrate it with real-time forecast weather, preferably at a high spatial resolution to allow site specific assessment. Implementation could be done electronically where large-scale systems would provide integrated weather forecasts, disease predictions and other relevant viticultural information. Implementation could also be done by the creation of a locked Excel® spreadsheet were an interested party could provide their own weather inputs or forecast inputs, and view how weather would change risk assessment.

One could argue that the data and knowledge gained from investigations in Chapters One and Two should be incorporated into the model presented in Chapter Three. While it would make a more complete model in terms of pathogen biology, I would argue that the addition of these submodels should only be done in terms of modification or justification of personal disease management decisions by a grower. Complexity and completeness do not always translate into usefulness, and I feel that the addition of submodels that incorporate the quantity of primary inoculum, or the reduced effect in very early stages of epidemic progress due to cold-induced disease resistance and damage to nascent colonies, would only lead to an additive effect of the variation already present in those submodels that could produce output of questionable reliability. The only exception to this would be the incorporation of a threshold value for the effects of acute cold temperature events during the very beginning stages of the growing season. This was not feasible in the New York model due to lack of annual variation in cold events, but could be developed in climates where the occurrence of such events is more variable year-to-year.

In conclusion, the work presented within this dissertation adds to the knowledge of *E. necator* biology and powdery mildew epidemic progress in the viticultural region of New York State. Investigations were aimed to fill knowledge gaps in this pathosytem, and to relate regional weather patterns to historical fruit disease severity in an effort to build a disease advisory system to aid in disease management decisions. The information provided herein fulfills these aims and will aid growers and academics in their future management and studies on grapevine powdery mildew.

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APPENDIX ONE

MONTE CARLO SIMILATIONS IN MICROSOFT EXCEL®

INTRODUCTION AND PROJECT DESCRIPTION

The use of a microcomputer spreadsheet for the development and execution of a Monte Carlo (1) simulation has many desirable attributes. First, it can be adapted to changes in model structure, quickly updated with new information, and it can be used by a broad and general audience.

There are many web-based tutorials on the set-up and execution of Monte Carlo simulations in computer spreadsheet and data programs such as Microsoft Excel® (2). One such website, www.vertex42.com has an excellent tutorial on Monte Carlo simulations basics, found at: http://www.vertex42.com/ExcelArticles/mc/MonteCarloSimulation.html, (accessed 18 June 2010), as well as downloadable example and template spreadsheets to help users become acquainted with the code language. The simulations discussed in Chapter Three were done in a spreadsheet derived with this tutorial help.

To use Monte Carlo simulation a deterministic model for predicting outcomes is needed. In the case of E_{to} calculations, this is was Equation 3.1 found in Chapter Three. Also needed are the maximum and minimum values for each of the equation input parameters, based on the site location data. For Chapter Three, this was the minimum and maximum values of solar radiation, wind speed, vapor pressure deficit, and average temperature for each calendar week (7 d) between 1 June and 31 July.

To begin the randomization process, these minima and maxima values need be entered into the spreadsheet. To create a random number between the minimum and maximum value for each parameter, the following formula was used:

= *min*+RAND()*(*max-min*)

where the *min* and *max* referred to the cells where the actual data was located. The formula looked like this:

=E14+RAND()*(F14-E14)

The RAND() function generated a new random number between 0 and 1 every time the spreadsheet was recalculated (the F9 key). Of course, for the Monte Carlo simulation, a large list (in the case of Chapter Three, n=5000) of random values can be generated for each input variable. This was done with the function listed above, but "$" was used before row and column designations to make Excel® refer back to a specific cell. Otherwise, Excel® would reference a spatially-corresponding cell when the equation was repeated within a column. An example of this specific referencing within a formula is below:

=E14+RAND()*(F14-E14)

and this action would be completed for each of the input variables. This formula generates a random number between the minimum and maximum value of the corresponding parameter. In the case for E_{to} calculation, there were also some input variables that were constant based on location, such as γ (psychrometric constant), and some variables that were dependent on temperature (such as the slope of the vapor pressure curve, Δ). For γ, 5000 cells within a column were filled with the same value. For Δ, a formula referencing the randomized temperature variable in that same row, was filled to 5000 cells within that column. The formula for Δ was:

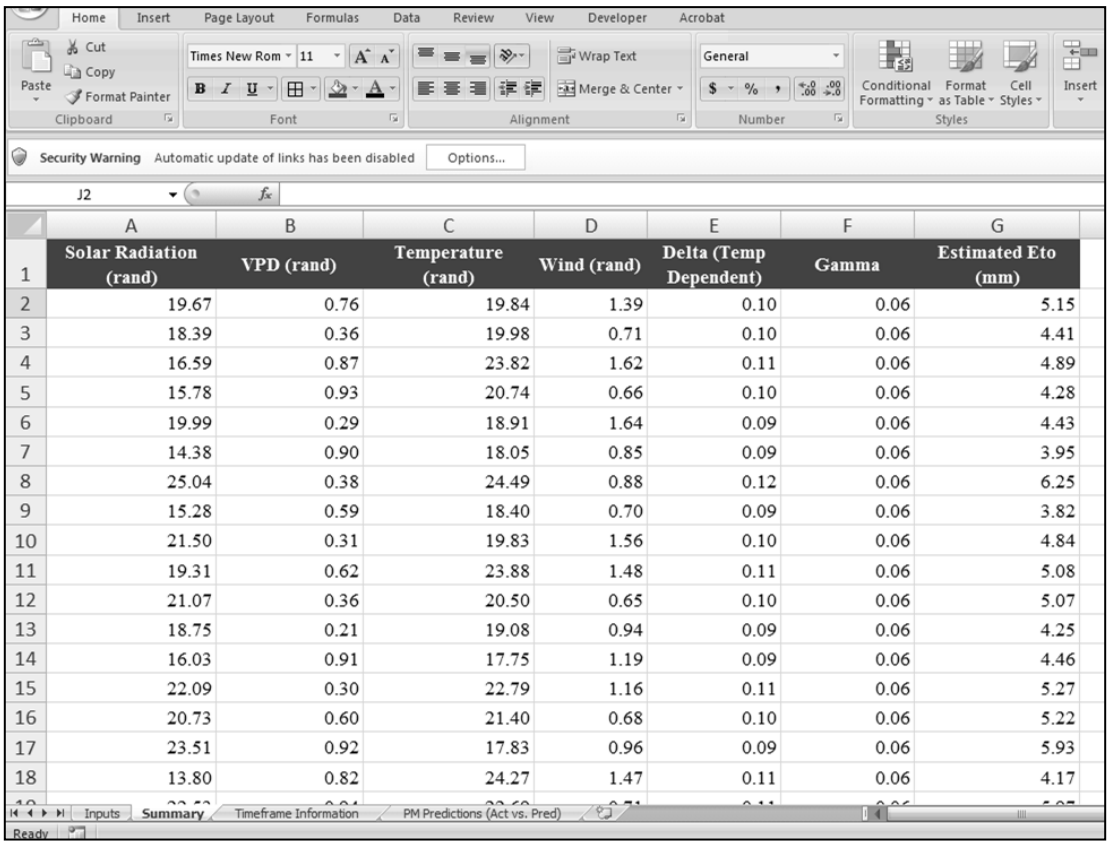
$$=(4098*(0.6108*(EXP((12.27*temp)/(temp+237.3)))))/(temp+237.3)^2$$

where *temp* refers to the randomized temperature variable. In a final column, E_{to} was calculated based on the randomized numbers that were generated, on a row by row basis. This was accomplished with the following equation:

$$=((0.408*\Delta*(SR))+(\gamma*(900/(temp+273)))*wind*vpd)/(\Delta+(\gamma*(1+0.34*wind)))$$

where *SR*, **γ,** *temp, wind, vpd* and Δ refer to their respective randomized (or constant value) cells. This equation was than filled to 5000 cells within a given column. A screen capture is seen in Fig. A.1.1, where each of the randomized and constant input parameters for the calculation of E_{to} are in their own respective columns. Data for the maximum and minimum values for each parameter were under a separate spreadsheet tab within the workbook.

Once the series of randomly generated E_{to} values were calculated, they were summarized and visualized in the Excel® spreadsheet. These values could also be easily exported to other programs for statistical analyses.



	A	B	C	D	E	F	G
1	Solar Radiation (rand)	VPD (rand)	Temperature (rand)	Wind (rand)	Delta (Temp Dependent)	Gamma	Estimated Eto (mm)
2	19.67	0.76	19.84	1.39	0.10	0.06	5.15
3	18.39	0.36	19.98	0.71	0.10	0.06	4.41
4	16.59	0.87	23.82	1.62	0.11	0.06	4.89
5	15.78	0.93	20.74	0.66	0.10	0.06	4.28
6	19.99	0.29	18.91	1.64	0.09	0.06	4.43
7	14.38	0.90	18.05	0.85	0.09	0.06	3.95
8	25.04	0.38	24.49	0.88	0.12	0.06	6.25
9	15.28	0.59	18.40	0.70	0.09	0.06	3.82
10	21.50	0.31	19.83	1.56	0.10	0.06	4.84
11	19.31	0.62	23.88	1.48	0.11	0.06	5.08
12	21.07	0.36	20.50	0.65	0.10	0.06	5.07
13	18.75	0.21	19.08	0.94	0.09	0.06	4.25
14	16.03	0.91	17.75	1.19	0.09	0.06	4.46
15	22.09	0.30	22.79	1.16	0.11	0.06	5.27
16	20.73	0.60	21.40	0.68	0.10	0.06	5.22
17	23.51	0.92	17.83	0.96	0.09	0.06	5.93
18	13.80	0.82	24.27	1.47	0.11	0.06	4.17

Figure A.1.1- Screen capture of an Excel® spreadsheet containing randomly generated E_{to} input parameters for the week of 21 July to 2 August for Geneva, NY used for Monte Carlo simulation.

A histogram of the distribution of E_{to} values was made, and simple functions that summarized the mean, median and mode of the 5000 values were also calculated using standard functions [=average(X:X), =median(X:X), =mode(X:X), respectively, where X:X refers to the cell range]. A graph of the simulation output of E_{to} calculations for the week of 15 June to 21 June is in Fig. A.1.2.

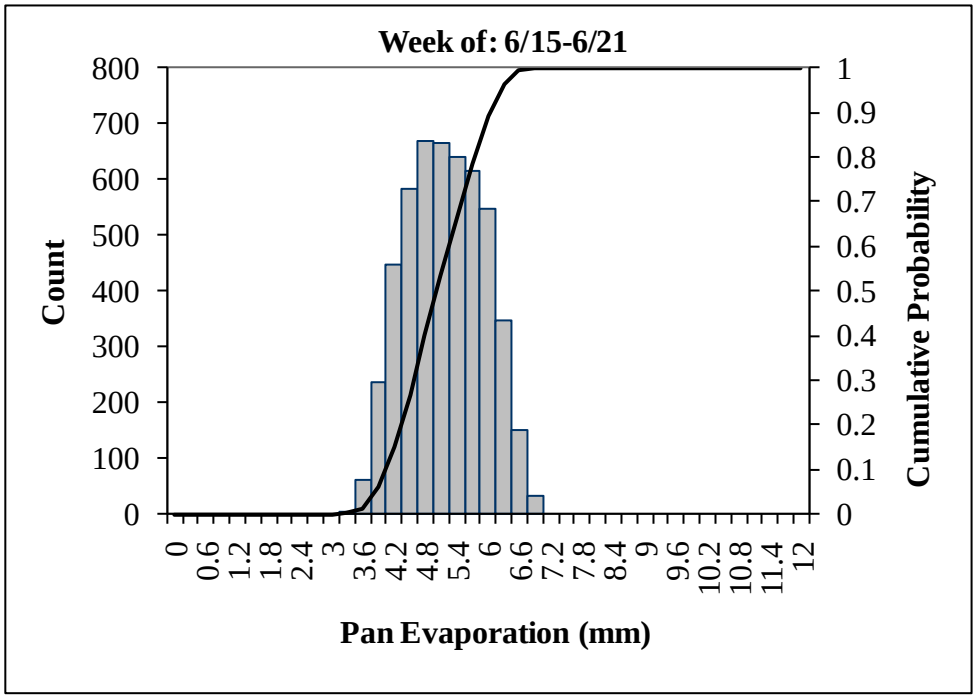


Figure A.1.2- Example output of simulated E_{to} using an Excel®-based Monte Carlo simulation. Histograms provide an excellent visualization of the output distribution, and cumulate curves can also be overlaid (pictured here) if additional visual cues are desired.

Quartile functions were useful for the summarization and comparison of the data. These functions were: [=Quartile(X:X,0) =Quartile(X:X,1), =Quartile(X:X,2), =Quartile(X:X,3), =Quartile(X:X,4)], computing the minimum value, 25th percentile, median value, 75th percentile, and maximum value, respectively. The Interquartile Range was the difference between the 75th and 25th percentile.

Probability functions also served as useful summary tools. To find the probability an individual value would be greater than a threshold, the following function was used:

=PERCENTRANK (X:X, *threshold*)

where X:X was the number array of the randomly generated E_{to} values, *threshold* was the value testing against. To find a value below a threshold level, the following modification of this function was used:

=1-PERCENTRANK (X:X, *threshold*)

Of course, this only resulted in a randomized calculation of E_{to} for a specified weekly time interval (based on your minimum and maximum input values). To create randomized values for all the weeks, the above process can be repeated for each weekly timeframe, or a series of conditional referencing statements (i.e. =if(*X,Y,Z*) can be used. Due to the complexity and individual nature of conditional statements, the statements used for in the Monte Carlo simulation of E_{to} distributions will not be discussed, and it is advised that the first few attempts using this type of simulation are done using separate spreadsheets for period where simulation is desired.

DISCUSSION

The use of commonly available microcomputer productivity software for data simulation has many advantages. Of which, the ability for mainstream usage and easy integration into other productively software are likely proponents which should make its use more widely acceptable among the scientific community. In the case of forecasting E_{to}, an individual user can modify and use the aforementioned Monte Carlo simulation on their personal computer, without the need of internet access or specialty programs to properly operate the simulation.

With this simulation specifically, the added benefit to designing a spreadsheet that can be manipulated by the user, is that forecasted maximum and minimum weather parameters can be substituted in for historical values, thus allowing for a

forecast of E_{to}. In the occurrence of unreliable or unpredictable forecast input data (i.e. wind speed and solar radiation), historical averages can still be used in combination with other forecasted inputs. This allows for the user to visualize how small changes in any of the input parameters influence predicted E_{to}, making it both an educational and a functional tool for biological understanding.

It was also easy to link the output from this spreadsheet into the Logistic Regression or Recursive Partitioning models presented in Chapter Three, to see the potential ranges of disease predictions with the historical or forecasted weather data to simulate E_{to}. This would only require a few additional columns for calculations under the input tab, and the creation of additional histograms relating to the output. This combination would be an even more powerful learning and teaching tool to see how small changes in weather input not only change E_{to} forecasts, but also disease severity forecasts.

In conclusion, the use of mainstream productivity software for disease modeling and forecasting, or the simulation of input parameters for disease forecasting is useful for a broad range of users. While not as powerful as some commercially available software, it seems to be a simple yet appropriate approach in developing systems that have the potential to be used by both researchers and growers alike. This approach provides a user-friendly interface for both scientific investigation, and personal inquiry at the grower level.

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APPENDIX TWO

VALIDATION THE NEW YORK POWDERY MILDEW RISK MODEL WITH HISTORICAL DISEASE DATA FROM THE RHEINHESSEN, GERMANY

INTRODUCTION AND PROJECT DESCRIPTION

As a means to test the robustness of the pan evaporation (E_{pan})-based New York Powdery Mildew Risk Assessment model described in Chapter Three, simulated weather data from Oppenheim, Germany from 1956-1989, and resultant risk assessments were compared to historical disease data from that region (2). Oppenheim, Germany was used for validation due to its similar climate, grapevine phenological development, and the availability of historical disease assessments.

Since historical disease severity ratings were dated earlier than the availability of reliable and complete weather data sets, the associated weather was simulated by ZedEx, Inc, Bellefonte, Pennsylvania, USA. Parameters simulated were maximum, minimum and average daily temperature; average relative humidity; total precipitation; average wind speed; solar radiation; and estimated evapotranspiration for grass. Solar radiation data was a compilation of existing measurements and educated assumptions based on historical cloud coverage due to the difficulties in accurately modeling it. Evapotranspiration of grass was included in the dataset, but evapotranspiration (E_{to}) was also calculated via the FAO modified Penman-Monteith equation (1) as discussed in Chapter Three. Cluster severity was not specifically described in the historical disease data used for validation, but it was inferred based on the qualitative descriptions of epidemics associated with the overall vine disease rating (2).

Average dates for key grape phenological stages were computed from data spanning 1990-2004 in Oppenheim, Germany (Table A.2.1). Phenology data were

from *Vitis vinifera* 'Müller-Thurgau'. Grapevine phenological development in the Rheinhessen region of Germany (Oppenheim included), and the Finger Lakes of New York, are similar on a calendar basis. This makes the Rheinhessen an ideal region for the comparison and validation of outputs for the New York Powdery Mildew Risk Assessment model.

Table A.2.1- Average grape phenological development for *Vitis vinifera* 'Müller-Thurgau' from 1990-2004 in Oppenheim, Germany.

Phenological State	Average Day
Budbreak	7-May
Full Bloom	14-Jun
Berry Set	30-Jun
Veraison	7-Aug
Harvest	27-Sep
Leaf Fall	26-Oct

To predict powdery mildew severity risk for fruit, degree day accumulation (base 10°C) was calculated for the previous autumn for each year, and average E_{to} from 1 June to 31 July of the current season was calculated from the simulated weather data and applied to the powdery mildew risk assessment models presented in Chapter Three. Actual disease severity ratings for an entire vineyard as described by Hill (2) for the Rheinhessen region varied from year to year (Table A.2.2). Degree day accumulation, calculated E_{to}, and the predicted risk for severe cluster infection using the New York Powdery Mildew Risk Assessment models were not very different from year-to-year (Table A.2.2). Both the recursive partitioning model and the logistic regression models discussed in Chapter Three were calculated. For the logistic regression model, a 'Severe' year was classified 'Severe' when the probability of a 'Severe' prediction was >0.29.

Table A.2.2- Powdery Mildew Risk predictions for Oppenheim, Germany.

Year	Disease Severity[x]	Autumn DD (10) 8/1-9/15	Calculated Eto (mm) 6/1-7/31	Recursive Partition Prediction	Logistic Regression Prediction
1956	0.5	410.93	4.58	60% Severe	Severe
1957	1.5	444.23	5.37	60% Severe	Severe
1958	1.5	607.14	4.96	Severe	Severe
1959	2.5	669.13	5.68	Severe	Severe
1960	2.0	536.24	5.19	Severe	Severe
1961	3.0	597.65	4.96	Severe	Severe
1962	0.5	633.85	5.41	Severe	Severe
1963	0.5	553.15	5.40	Severe	Severe
1964	0.5	637.48	5.83	Severe	Severe
1965	0.5	485.43	5.05	Severe	Severe
1966	1.0	572.63	5.01	Severe	Severe
1967	1.5	549.29	5.26	Severe	Severe
1968	0.5	552.99	5.19	Severe	Severe
1969	0.5	592.29	5.13	Severe	Severe
1970	0.5	628.99	5.24	Severe	Severe
1971	0.5	594.74	5.13	Severe	Severe
1972	0.5	497.66	5.06	Severe	Severe
1973	1.0	753.53	5.48	Severe	Severe
1974	0.5	645.99	5.06	Severe	Severe
1975	1.0	690.01	5.44	Severe	Severe
1976	0.5	597.70	6.33	Mild	Severe
1977	1.0	559.81	5.25	Severe	Severe
1978	2.5	517.37	5.03	Severe	Severe
1979	0.0	551.74	5.43	Severe	Severe
1980	1.0	580.59	4.76	Severe	Severe
1981	3.0	567.90	4.95	Severe	Severe
1982	1.0	625.63	5.59	Severe	Severe
1983	2.5	653.38	5.75	Severe	Severe
1984	0.0	578.51	5.08	Severe	Severe
1985	0.5	514.63	5.18	Severe	Severe
1986	0.0	505.23	5.30	Severe	Severe
1987	1.0	553.27	4.85	Severe	Severe
1988	3.0	591.12	5.01	Severe	Severe
1989	3.0	573.25	5.24	Severe	Severe

[x] Disease severity scale is as: 0- No disease symptoms; 1-First symptoms on leaves after veraison; 2-Fruit symptoms 14 d preveraison; 3- Severe fruit infection immediately after flowering (2).

Average E_{to} value for each of the disease rating categories were not statistically significantly different from each other (Tukey's HSD, $P>0.05$), but there was a trend towards higher E_{to} values in low disease years (Table A.2.3). There were some cases, however, (e.g. the 2.5 rating category) where high E_{to} values were associated with high levels of disease. However, since there are two known sources of primary inoculum in the region, cleistothecia and flag shoots, the high disease ratings could be a result of the presence of flag shoots, which provide an early source of secondary inoculum for cluster infection.

Table A.2.3- Average E_{to} values for years within each disease rating category.

Disease Rating	Average E_{to} 6/1-7/31
0.0	5.27 (0.20)
0.5	5.28 (0.09)
1.0	5.19 (0.12)
1.5	5.12 (0.17)
2.0	5.19 (0.34)
2.5	5.49 (0.20)
3.0	5.04 (0.17)

DISCUSSION

Calculated E_{to} for the Rheinhessen region of Germany was significantly lower than that of the Finger Lakes region of New York State (average E_{to} of 5.23 ±0.06 mm for Germany versus 5.86 ±0.09 mm for New York, $P<0.0001$ Student's *t*-test). Autumn degree day accumulation for 1 Aug to 15 Sept was significantly higher in the Rheinhessen (580.49 ±9.49 for Germany versus 435.00 ±12.52 for New York State, $P<0.0001$, Student's *t*-test). This combination of warmer autumn temperatures, and reduced evaporation potential biases the New York Powdery Mildew Risk Assessment model for a 'Severe' prediction when deployed in the Rheinhessen. However, the

observed disease severity data came with a few caveats. First, the vineyards where the observations were made were in research plots, which were under an annual no-spray program. While disease pressure was high (from both cleistothecia and flag shoot inoculum sources), the vines were of reduced vigor. Secondly, the data was not representative of disease pressure for the entire region. Finally, there was likely some error in inferring cluster disease severity from the whole-vine rating scale used.

Topography of the Rheinhessen region can also make regional evapotranspiration generalizations challenging. The Rheinhessen region is descriptively hilly. The calculation of E_{to} is influenced by such topography: solar radiation intensity is influenced by angle to the sun, wind speeds can vary with increased barriers, temperatures can develop gradients along such slopes, and elevation can influence atmospheric pressure. This suggests that if a model like that presented in Chapter 3 were to be adopted for a region like the Rheinhessen, or specifically for Oppenheim, Germany, E_{to} would need to be modeled on a site-specific scale to take into account these variations.

Another adaptation that would need to be made is in regards to the calendar day thresholds in the model. Currently, these are based on key developmental stages for the grapevine in New York State. The previous autumn heat accumulation, defined as 1 Aug to 15 Sept, approximately aligns with the end of seasonal spray programs and harvest. While harvest dates appear to be similar between the two regions, pre-harvest intervals for fungicide applications may be drastically different. The timeframe for E_{to} calculation is 1 June to 31 July, which in New York, aligns with 2 wks prebloom to peasize fruit development. This timeframe is almost identical to that of the Rheinhessen, but E_{to} calculation modifications mentioned above should be considered.

As seen in Chapter Three, calculated E_{to} tended to underestimate observed E_{pan}. Since the model thresholds were based on observed data, prediction accuracy may be improved by using collected E_{pan} data rather than calculating E_{to}.

In conclusion, the use of an E_{pan}-based model for powdery mildew risk assessment has the potential for use in other climates, but site modifications must be considered. This model was designed in an area that lacks flag shoot development, which can provide high disease severity foci without regional epidemics. In addition, the model was based on observed E_{pan} data, which though widely available, is not always reported or measured due to lack of need in areas with regular rainfall. Despite these drawbacks, this model does have the potential to describe environmental favorability for powdery mildew development in regions outside of New York State.

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APPENDIX THREE

SCREENING WEATHER DATA FOR CORRELATION TO POWDERY MILDEW SEVERITY ON GRAPE CLUSTERS: METHODS AND WEATHER PARAMETERS USED IN EARLY MODEL DEVELOPMENT

INTRODUCTION AND RATIONAL

The development of the New York Powdery Mildew Risk Assessment Model presented in Chapter Three was not as straight forward as it would appear in final presentation; the input parameters of E_{pan} and Late Summer Degree Day accumulation were the result of intensive data mining and screening for association to powdery mildew severity on the *Vitis vinifera* hybrid 'Rosette' clusters grown in Geneva, NY, USA, as previously described in the Materials and Methods section of Chapter Three. Presented here are different weather variables that ***did not*** provide significant or informative correlation to cluster disease severity for model development. This information is presented to help with future research endeavors that involve powdery mildew epidemic predictions based on weather.

Weather inputs for model development were screened for correlation to log transformed cluster disease severity values via regression (Table A3.1). These inputs included average daily net solar radiation (SR), cumulative daily net SR, average daily relative humidity (RH), average daily maximum RH, average daily minimum RH, average daily temperature, average daily maximum temperature, average daily minimum temperature, degree day accumulation (base 0 and 10°C), cumulative rain events (base 2.5 mm) either associated with set temperatures (>10°C) or not, average daily vapor pressure deficit (VPD), cumulative daily VPD, and as mentioned in Chapter Three, pan evaporation (E_{pan}).

Table A.3.1- Examples of the correlation between weather parameters at specified timeframes and log transformed cluster disease severity on *Vitis vinifera* hybrid 'Rosette' in Geneva, New York, USA for the years 1985-2007 (n=23). These weather inputs did not provide a high level of explanatory power for the development of the Powdery Mildew Risk Assessment Model.

Value	Timeframe	R^2
Average Daily Solar Radiation (Taken as the average of the daily solar radiation over the period specified.)	5/1-7/31	0.13
	6/1-6/30	0.04
	BL-3wkpBL*	0.0003
Average Temperature (Taken as the average of the average daily temperature over the period specified.)	5/1-7/31	0.21
	6/1-6/30	0.02
	BL-3wkpBL*	0.01
Average Temperature (Maximum) (Taken as the average of the maximum daily temperature over the period specified.)	5/1-7/31	0.30
	6/1-6/30	0.06
	BL-3wkpBL*	0.01
Average Temperature (Minimum) (Taken as the average of the minimum daily temperature over the period specified.)	5/1-7/31	0.07
	6/1-6/30	0.003
	BL-3wkpBL*	0.09
Degree Day (10°C) Accumulation (Taken as the degree day base 10°C from the average daily temperature over the period specified.)	6/1-7/31	0.13
Average Maximum Relative Humidity (Taken as the average of the maximum daily relative humidity over the period specified.)	6/1-7/31	0.31
Average Minimum Relative Humidity (Taken as the average of the minimum daily relative humidity over the period specified.)	6/1-7/31	0.36
Average Vapor Pressure Deficit (Taken as the average of the daily vapor pressure deficit over the period specified.)	5/1-7/31	0.40
	6/1-6/30	0.51
	BL-3wkpBL*	0.34

*BL-3wkpBL = Bloom to 3 weeks postbloom as defined by recorded phenology.

These weather inputs were also screened over different time periods, including both phenological and calendar-based timeframes (Table A3.2). Timeframes for in-season weather analysis were biased towards the periods of (i) vine emergence, or (ii) cluster emergence and subsequent duration of peak cluster susceptibility to powdery mildew infection (4, 5).

Table A.3.2- Complete list of timeframes used in analysis of in-season weather inputs for the development of the Powdery Mildew Risk Assessment Model presented in Chapter Three.

Time Frame	Description/Timeframe Justification
1 May -31 July	Period of historical budbreak to the complete onset of ontogenic resistance.
1 May- 30 June	Period of historical budbreak to fruit set. This is the period of first available host tissue through the peak period of cluster susceptibility to powdery mildew.
1 June- 31 July	Period of historical cluster emergence to the complete onset of ontogenic resistance.
1 June-30 June	Period of historical cluster emergence to fruit set. This is traditionally the peak period of cluster susceptibility to powdery mildew.
Budbreak- Bloom	Recorded phenological dates. Missing budbreak and bloom dates were estimated as 1 May and 15 June, respectively. This is the period of initial infection through beginning of cluster susceptibility to powdery mildew.
Budbreak- 3 weeks postbloom	Recorded phenological dates. Missing budbreak and bloom dates were estimated as 1 May and 15 June, respectively. This is the period of initial infection through the peak period of cluster susceptibility to powdery mildew.
1 week prebloom- 2 weeks postbloom	Recorded phenological dates. Missing budbreak and bloom dates were estimated as 1 May and 15 June, respectively. This is a focused window around the period of maximum cluster exposure and peak cluster susceptibility to powdery mildew.
1 week prebloom- 1 week postbloom	Recorded phenological dates. Missing budbreak and bloom dates were estimated as 1 May and 15 June, respectively. This is a focused, truncated window around the period of peak cluster susceptibility to powdery mildew.
Bloom- 3 weeks postbloom	Recorded phenological dates. Missing budbreak and bloom dates were estimated as 1 May and 15 June, respectively. This is a focused window around the period of peak cluster susceptibility, emphasizing later cluster infections.
Bloom- 1 week postbloom	Recorded phenological dates. Missing budbreak and bloom dates were estimated as 1 May and 15 June, respectively. This is a focused, truncated window around the period of peak cluster susceptibility, emphasizing later cluster infection.

In addition to average and cumulative values, "severity scores" were also developed for VPD, SR, E_{pan} and temperature (Table A3.3). Scores were based on

estimated thresholds which were observable when plotting daily values against calendar date, for "Mild" and "Severe" powdery mildew years (as described in Chapter Three). Temperature was the exception, with threshold values based on temperature response curves previously described (3). Threshold values of VPD, SR, E_{pan} and temperature were then ranked, from 1 to 3 ("Mild", "Intermediate" and "Severe", respectively), and score accumulations were computed for the aforementioned timeframes. Score combinations were also used for each day, and accumulated scores were computed as previously mentioned. Score values were assessed for a relationship to cluster disease severity. Higher score accumulation, in theory, would correlate to higher disease severity.

Table A.3.3- Weather parameter threshold values used to accumulate "severity" values over specified time intervals during the growing season. Thresholds were established using the years 1986, 1988, 1989, 1991-93, 2001 and 2003, which represented severe and mild years for powdery mildew severity on clusters on *Vitis vinifera* hybrid 'Rosette' grown in Geneva, NY.

Weather Input	Category Thresholds		
	Mild (1)	Intermediate (2)	Severe (3)
Temperature	<15, >32°C	15 to 25°C	26 to 32°C
Vapor Pressure Deficit	>1 kPa	0.5 to 1 kPa	<0.5 kPa
Solar Radiation	>25 mJ/m^2	15-25 mJ/m^2	<15 mJ/m^2
Pan Evaporation	>5.0 mm		<5.0 mm

The scoring method, however, did not provide a more rapid assessment of the data compared to the use of raw data values. However, the threshold values may have potential in future model development or refinement of a rule-of-thumb for advising system. No statistical analyses beyond basic regression were performed comparing cumulative severity scores to actual cluster disease severity.

DISCUSSION

Use of basic weather inputs for the design of a grapevine powdery mildew model is a challenge when the model is built for a climate that is highly conducive for pathogen and disease development. There are likely reasons for this: (i) when dealing with an obligate biotroph, conditions that favor or discourage host development are also important, and (ii) climate conditions in regions such as upstate New York rarely reach the extreme values that would be pathogen- or host-limiting during the growing season. If a model was built around extreme thresholds that limit pathogen development, then it biases weather assessments (places undo importance on their role in epidemic development) for those extreme values, and ignores weather patterns that favor pathogen development. Single input parameters cannot capture the complex interactions that make up the daily weather conditions. Based on the results presented here, the use of individual weather parameters for a New York grapevine powdery mildew model did not provide useful information for decision management.

While SR, VPD, RH and temperature all have significant influences on pathogen development (1-3), the affects of their interaction on both the pathogen and the host combined is challenging to define. These were important parameters to consider in the process of model development, but they did not provide sufficient power to explain why certain years appear to be highly conducive for disease development on clusters. The only exception was VPD, which did have a high explanatory powdery when only considering 'Mild' and 'Severe' disease years, but broke down considerably when all years (1985-2007) were used for model refinement. However, exploration of these different parameters in model development was not a wasted exercise as it led to the use of E_{pan}, which integrates the aforementioned variables. The final result was the use of E_{pan} as a basis for the New York Powdery Mildew Risk Assessment Model presented in Chapter Three.

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